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Dear Sir:

Enclosed is a certified copy of the priority document for the above-identified application. Applicants hereby claim priority under 35 U.S.C. §§ 119 and/or 120 from the following application:

GB Patent Application No. 0209884.6 filed April 30, 2002.

Acknowledgment of the claim of priority and receipt of the certified copy is requested.

Respectfully submitted,  
FROMMER LAWRENCE & HAUG LLP

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ARES TRADING SA  
CHATEAU DE VAUMARCUS  
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SWITZERLAND

6275622001

Patents ADP number (if you know it)

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SWITZERLAND

4. Title of the invention

PROTEINS

5. Name of your agent (if you have one)

Carpmaels &amp; Ransford

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## PROTEINS

This invention relates to novel proteins (termed INSP052 and INSP055), herein identified as immunoglobulin domain-containing cell surface recognition molecules and to the use of these proteins and nucleic acid sequences from the encoding genes in the diagnosis, 5 prevention and treatment of disease.

All publications, patents and patent applications cited herein are incorporated in full by reference.

## BACKGROUND

The process of drug discovery is presently undergoing a fundamental revolution as the era 10 of functional genomics comes of age. The term "functional genomics", applies to an approach utilising bioinformatics tools to ascribe function to protein sequences of interest. Such tools are becoming increasingly necessary as the speed of generation of sequence data is rapidly outpacing the ability of research laboratories to assign functions to these protein sequences.

15 As bioinformatics tools increase in potency and in accuracy, these tools are rapidly replacing the conventional techniques of biochemical characterisation. Indeed, the advanced bioinformatics tools used in identifying the present invention are now capable of outputting results in which a high degree of confidence can be placed.

Various institutions and commercial organisations are examining sequence data as they 20 become available and significant discoveries are being made on an on-going basis. However, there remains a continuing need to identify and characterise further genes and the polypeptides that they encode, as targets for research and for drug discovery.

Recently, a remarkable tool for the evaluation of sequences of unknown function has been developed by the Applicant for the present invention. This tool is a database system, 25 termed the Biopendium search database, that is the subject of WO 01/69507. This database system consists of an integrated data resource created using proprietary technology and containing information generated from an all-by-all comparison of all available protein or nucleic acid sequences.

The aim behind the integration of these sequence data from separate data resources is to 30 combine as much data as possible, relating both to the sequences themselves and to information relevant to each sequence, into one integrated resource. All the available data

relating to each sequence, including data on the three-dimensional structure of the encoded protein, if this is available, are integrated together to make best use of the information that is known about each sequence and thus to allow the most educated predictions to be made from comparisons of these sequences. The annotation that is generated in the database and 5 which accompanies each sequence entry imparts a biologically relevant context to the sequence information.

This data resource has made possible the accurate prediction of protein function from sequence alone. Using conventional technology, this is only possible for proteins that exhibit a high degree of sequence identity (above about 20%-30% identity) to other 10 proteins in the same functional family. Accurate predictions are not possible for proteins that exhibit a very low degree of sequence homology to other related proteins of known function.

### **SIGNAL PEPTIDE-CONTAINING PROTEINS**

The ability of cells to make and secrete extracellular proteins is central to many biological 15 processes. Enzymes, growth factors, extracellular matrix proteins and signaling molecules are all secreted by cells. This is through fusion of a secretory vesicle with the plasma membrane. In most cases, but not all, proteins are directed to the endoplasmic reticulum and into secretory vesicles by a signal peptide. Signal peptides are cis-acting sequences that affect the transport of polypeptide chains from the cytoplasm to a membrane bound 20 compartment such as a secretory vesicle. Polypeptides that are targeted to the secretory vesicles are either secreted into the extracellular matrix or are retained in the plasma membrane. The polypeptides that are retained in the plasma membrane will have one or more transmembrane domains. Examples of signal peptide containing proteins that play a central role in the functioning of a cell are cytokines, hormones, extracellular matrix 25 proteins, adhesion molecules, receptors, proteases, and growth and differentiation factors.

### **IMMUNOGLOBULIN DOMAIN-CONTAINING CELL SURFACE RECOGNITION MOLECULES**

Immunoglobulin domain-containing cell surface recognition molecules have been shown to play a role in diverse physiological functions, many of which can play a role in disease 30 processes. Alteration of their activity is a means to alter the disease phenotype and as such identification of novel immunoglobulin domain-containing cell surface recognition molecules is highly relevant as they may play a role in many diseases, particularly

inflammatory disease, oncology, and cardiovascular disease. Immunoglobulin domain-containing cell surface recognition molecules are involved in a range of biological processes, including: embryogenesis (Martin-Bermudo, M.D. *et al.*, *Development*. 2000 127(12):2607-15; Chen, L.M., *et al.*, *J Neurosci*. 2000 20(10):3776-84; Zweegman, S., *et al.*,

5 *Exp Hematol*. 2000 28(4):401-10; Darribere, T., *et al.*, *Biol Cell*. 2000 92(1):5-25), maintenance of tissue integrity (Eckes, B., *et al.*, *J Cell Sci*. 2000 113(Pt 13):2455-2462; Buckwalter, J.A., *et al.*, *Instr Course Lect*. 2000 49:481-9; Frenette, P.S., *et al.*, *J Exp Med*. 2000 191(8):1413-22; Delmas, V., *et al.*, *Dev Biol*. 1999 216(2):491-506; Humphries, M.J., *et al.*, *Trends Pharmacol Sci*. 2000 21(1):29-32; Miosge, N., *et al.*, *Lab Invest*. 1999 10 79(12):1591-9; Nagaoka T, *et al.* *Am J Pathol* 2000 Jul 157:1 237-47; Nwariaku FE, *et al.* *J Trauma* 1995 39(2): 285-8; Zhu X, *et al.* *Zhonghua Zheng Xing Shao Shang Wai Ke Za Zhi* 1999 15(1): 53-5), leukocyte extravasation/inflammation (Lim, L.H., *et al.* *Am J Respir Cell Mol Biol*. 2000 22(6):693-701; Johnston, B., *et al.*, *Microcirculation*. 2000 7(2):109-18; Mertens, A.V., *et al.*, *Clin Exp Allergy*. 1993 23(10):868-73; Chcialowski, A., *et al.*, *Pol Merkuriusz Lek*. 2000 7(43):13-7; Rojas, A.I., *et al.*, *Crit Rev Oral Biol Med*. 1999 10(3):337-58; Marinova-Mutafchieva, L., *et al.*, *Arthritis Rheum*. 2000 43(3):638-44; Vijayan, K.V., *et al.*, *J Clin Invest*. 2000 105(6):793-802; Currie, A.J., *et al.*, *J Immunol*. 2000 164(7):3878-86; Rowin, M.E., *et al.*, *Inflammation*. 2000 24(2):157-73; Johnston, B., *et al.*, *J Immunol*. 2000 164(6):3337-44; Gerst, J.L., *et al.*, *J Neurosci Res*. 2000 59(5):680-4; Kagawa, T.F., *et al.*,

20 *Proc Natl Acad Sci U S A*. 2000 97(5):2235-40; Hillan, K.J., *et al.*, *Liver*. 1999 19(6):509-18; Panes, J., 1999 22(10):514-24; Arao, T., *et al.*, *J Clin Endocrinol Metab*. 2000 85(1):382-9; Souza, H.S., *et al.*, *Gut*. 1999 45(6):856-63; Grunstein, M.M., *et al.*, *Am J Physiol Lung Cell Mol Physiol*. 2000 278(6):L1154-63; Mertens, A.V., *et al.*, *Clin Exp Allergy*. 1993 23(10):868-73; Berends, C., *et al.*, *Clin Exp Allergy*. 1993 23(11):926-33; Fernvik, E., *et al.*,

25 *Inflammation*. 2000 24(1):73-87; Bocchino, V., *et al.*, *J Allergy Clin Immunol*. 2000 105(1 Pt 1):65-70; Jones SC, *et al.*, *Gut* 1995 36(5):724-30; Liu CM, *et al.*, *Ann Allergy Asthma Immunol* 1998 81(2):176-80; McMurray RW *Semin Arthritis Rheum* 1996 25(4):215-33; Takahashi H, *et al* *Eur J Immunol* 1992 22(11): 2879-85; Carlos T, *et al* *J Heart Lung Transplant* 1992 11(6): 1103-8; Fabrega E, *et al.*, *Transplantation* 2000 69(4): 569-73;

30 Zohrens G, *et al*, *Hepatology* 1993 18(4): 798-802; Montefort S, *et al.* *Am J Respir Crit Care Med* 1994 149(5): 1149-52), oncogenesis (Orr, F.W., *et al.*, *Cancer*. 2000 88(S12):2912-2918; Zeller, W., *et al.*, *J Hematother Stem Cell Res*. 1999 8(5):539-46; Okada, T., *et al.*, *Clin Exp Metastasis*. 1999 17(7):623-9; Mateo, V., *et al.*, *Nat Med*. 1999 5(11):1277-84;

Yamaguchi, K., *et al.*, J Exp Clin Cancer Res. 2000 19(1):113-20; Maeshima, Y., *et al.*, J Biol Chem. 2000 275(28):21340-8; Van Waes, C., *et al.*, Int J Oncol. 2000 16(6):1189-95; Damiano, J.S., *et al.*, Leuk Lymphoma. 2000 38(1-2):71-81; Seftor, R.E., *et al.*, Cancer Metastasis Rev. 1999 18(3):359-75; Shaw, L.M., J Mammary Gland Biol Neoplasia. 1999 5 4(4):367-76; Weyant, M.J., *et al.*, Clin Cancer Res. 2000 6(3):949-56), angiogenesis (Koch AE, *et al* Nature 1995 376 (6540): 517-9; Wagener C & Ergun S. Exp Cell Res 2000 261(1): 19-24; Ergun S, *et al.* Mol Cell 2000 5(2): 311-20), bone resorption (Hartman GD, & Duggan ME. Expert Opin Investig Drugs 2000 9(6): 1281-91; Tanaka Y, *et al.* J Bone Miner Res 1995 10(10): 1462-9; Lark MW, *et al.* J Pharmacol Exp Ther 1999 291(2): 612-7; Raynal C, 10 10 *et al.* Endocrinology 1996 137(6):2347-54; Ilvesaro JM, *et al.* Exp Cell Res 1998 242(1): 75-83), neurological dysfunction (Ossege LM, *et al.* Int Immunopharmacol 2001 1:1085-100; Bitsch A, *et al*, Stroke 1998 29:2129-35; Iadecola C & Alexander M. Curr Opin Neurol 2001 14:89-94; Becker K, *et al* Stroke 2001 32(1): 206-11; Relton JK, *et al* Stroke 2001 32(1): 199-205; Hamada Y, *et al* J Neurochem 1996 66:1525-31), thrombogenesis (Wang, Y.G., *et al.*, J 15 Physiol (Lond). 2000 526(Pt 1):57-68; Matsuno, H., *et al.*, Nippon Yakurigaku Zasshi. 2000 115(3):143-50; Eliceiri, B.P., *et al.*, Cancer J Sci Am. 2000 6(Suppl 3):S245-9; von Beckerath, N., *et al.*, Blood. 2000 95(11):3297-301; Topol, E.J., *et al.*, Am Heart J. 2000 139(6):927-33; Kroll, H., *et al.*, Thromb Haemost. 2000 83(3):392-6), and invasion/adherence of bacterial pathogens to the host cell (Dersch P, *et al.* EMBO J 1999 18(5): 1199-1213).

20 The detailed characterisation of the structure and function of several immunoglobulin-domain containing cell surface recognition molecule families has led to active programs by a number of pharmaceutical companies to develop modulators for use in the treatment of diseases involving inflammation, oncology, neurology, immunology and cardiovascular function. Immunoglobulin domain containing cell surface recognition molecules are 25 involved in virtually every aspect of biology from embryogenesis to apoptosis. They are essential to the structural integrity and homeostatic functioning of most tissues. It is therefore not surprising that defects in immunoglobulin domain containing cell surface recognition molecules cause disease and that many diseases involve modulation of immunoglobulin domain containing cell surface recognition molecule function. The members of this family 30 are described below in Table 1.

The Immunoglobulin domain containing cell surface recognition molecule family in fact contains several distinct families. Of these families, some are of particular pharmaceutical interest due to small molecule tractability. They include:

1. The immunoglobulin adhesion molecules represent the counter receptors for the integrins and includes the intracellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs). Members are composed of variable numbers of globular, immunoglobulin-like, extracellular domains. Some members of the family, for example, 5 PECAM-1 (CD31) and NCAM, mediate homotypic adhesion. Some members of the family, for example ICAM-1 and VCAM-1, mediate adhesion via interactions with integrins.

2. Cell surface growth factor receptors. Growth factors are extracellular and in order to exert a biological effect they interact with specific, high affinity receptors located on the 10 plasma membranes of target cells. The molecular characterisation of a variety of different growth factor receptors revealed that they fall into defined families; the tyrosine kinase receptors, G-protein associated seven transmembrane receptors, and the serine/threonine kinase receptors. The tyrosine kinase receptors are characterised by an extracellular domain, a transmembrane domain, and an intracellular domain which 15 possess tyrosine kinase activity. VEGFR, PDGFR, FGFR, CSF-1R and c-KIT are examples of tyrosine kinase growth factor receptors which also contain immunoglobulin domains in the extracellular portion. Dys-regulation of growth factor function results in many different disease phenotypes, including, but not exclusive to oncology (Bartucci M *et al.*, (2001) *Cancer Res.* Sep 15;61(18):6747-54, Dias S *et al.*, (2001) *Proc Natl Acad Sci U S A.* Sep 11;98(19):10857-62, Djavan B *et al.*, (2001) *World J Urol.* 19(4):225-33), inflammation (Fiocchi C. (2001) *J Clin Invest.* Aug;108(4):523-6, Hodge S *et al.*, (2001) *Respirology.* Sep;6(3):205-211, Fenwick SA *et al.*, (2001) *J Anat.* Sep;199(Pt 3):231-40), neurological (Cooper JD *et al.*, (2001) *Proc Natl Acad Sci U S A* 98(18):10439-44, Fahnestock M *et al.*, (2001) *Mol Cell Neurosci* 18(2):210-20, and metabolism (Vickers MH *et al.*, (2001) *Endocrinology.* 142(9):3964-73).

**Table 1: Immunoglobulin domain-containing cell surface recognition molecules**

<b>Receptor</b>	<b>Ligand</b>	<b>Distribution</b>
ICAM-1 5 Ig domains	LFA-1 (CD11a/CD18) Mac-1 (CD11b/CD18), CD43	Widespread, endothelial cells, fibroblasts, epithelium, monocytes, lymphocytes, dendritic cells, chondrocytes.
ICAM-2	LFA-1 (CD11b)	endothelial cells (high): lymphocytes, monocytes,

2 Ig domains		basophils, platelets (low).
ICAM-3 5 Ig domains	LFA-1 ( $\alpha$ d/CD18)	Lymphocytes, monocytes, neutrophils, eosinophils, basophils.
VCAM-1 6 or 7 Ig domains	$\alpha$ 4 $\beta$ 1, $\alpha$ 4 $\beta$ 7	Endothelial cells, monocytes, fibroblasts, dendritic cells, bone marrow stromal cells, myoblasts.
LFA-3 6 Ig domains	CD2	Endothelial cells, leukocytes, epithelial cells
PECAM-1 (CD31)	CD31, heparin	Endothelial cells (at EC-EC junctions), T cell subsets, platelets, neutrophils, eosinophils, monocytes, smooth muscle cells, bone marrow stem cells.
NCAM	NCAM, heparin SO <sub>4</sub>	Neural cells, muscle
MAdCAM-1 4 Ig domains	$\alpha$ 4 $\beta$ 7, L-selectin	Peyer's patch, mesenteric lymph nodes, mucosal endothelial cells, spleen.
CD2	CD58, CD59, CD48	T lymphocytes
VEGFR	VEGF	Widespread, retina, umbilical vein, adrenal, NT2 neuronal precursor cells
FGFR	FGF	Widespread, brain, colon, ovary
KIT	Stem Cell Factor, MGF	Widespread, foetus, melanocytes, gall bladder, cerebellum, gastric epithelium (low)
PDGFR	PDGF	Widespread, breast, placenta, fibroblast, lung, ovary, skin, heart
CSF-1R	CSF	Widespread, placenta, liver, multiple sclerosis lesions, spleen, lung, breast.

Immunoglobulin domain-containing cell surface recognition molecules have thus been shown to play a role in diverse physiological functions, many of which can play a role in disease processes. Alteration of their activity is a means to alter the disease phenotype and 5 as such identification of novel Immunoglobulin domain-containing cell surface recognition molecules is highly relevant as they may play a role in many diseases, particularly immunology, inflammatory disease, oncology, cardiovascular disease, central nervous system disorders and infection.

## THE INVENTION

10 The invention is based on the discovery that the INSP052 and INSP055 proteins function

as immunoglobulin domain-containing cell surface recognition molecules.

In one embodiment of the first aspect of the invention, there is provided a polypeptide which:

- (i) comprises or consists of the amino acid sequence as recited in SEQ ID NO:2, SEQ 5 ID NO:4, SEQ ID NO: 6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, or SEQ ID NO:16;
- (ii) is a fragment thereof having the activity of a polypeptide according to (i), or having an antigenic determinant in common with a polypeptide according to (i); or
- (iii) is a functional equivalent of (i) or (ii).

10 By "the activity of a polypeptide according to (i)", we refer to immunoglobulin domain-containing cell surface recognition molecule activity.

The polypeptide having the sequence recited in SEQ ID NO:2 is referred to hereafter as "the INSP052 exon 1 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:4 is referred to hereafter as "the INSP052 exon 2 polypeptide". The polypeptide 15 having the sequence recited in SEQ ID NO:6 is referred to hereafter as "the INSP052 exon 3 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:8 is referred to hereafter as "the INSP052 exon 4 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:10 is referred to hereafter as "the INSP052 exon 5 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:12 is referred to hereafter as "the 20 INSP052 exon 6 polypeptide". The polypeptide having the sequence recited in SEQ ID NO:14 is referred to hereafter as "the INSP052 exon 7 polypeptide". Combining SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 produces the sequence recited in SEQ ID NO:16. The polypeptide having the sequence recited in SEQ ID NO:16 is referred to hereafter as the INSP052 polypeptide.

25 The term "INSP052 exon polypeptides" as used herein includes polypeptides comprising or consisting of the INSP052 exon 1 polypeptide, the INSP052 exon 2 polypeptide, the INSP052 exon 3 polypeptide, the INSP052 exon 4 polypeptide, the INSP052 exon 5 polypeptide, the INSP052 exon 6 polypeptide, the INSP052 exon 7 polypeptide or the INSP052 polypeptide.

30 In one embodiment, the polypeptide according to this embodiment consists of the amino acid sequence recited in SEQ ID NO:16 or is a fragment of or functional equivalent

thereof. In another embodiment, the polypeptide consists of the amino acid sequence recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14, or a variant thereof.

In a second embodiment of the first aspect of the invention, there is provided a polypeptide  
5 which:

- (i) comprises or consists of the amino acid sequence as recited in SEQ ID NO:18,
- (ii) is a fragment thereof having the activity of a polypeptide of (i), or having an antigenic determinant in common with a polypeptide of (i); or
- (iii) is a functional equivalent of (i) or (ii).

10 By "the activity of a polypeptide according to (i)", we refer to immunoglobulin domain-containing cell surface recognition molecule activity.

Preferably, the polypeptide according to this embodiment consists of the amino acid sequence recited in SEQ ID NO:18 or is a fragment of or functional equivalent thereof.

The polypeptide having the sequence recited in SEQ ID NO:18 is referred to hereafter as  
15 "the INSP055 polypeptide".

In a second aspect, the invention provides a purified nucleic acid molecule which encodes a polypeptide of the first aspect of the invention.

Preferably, the purified nucleic acid molecule comprises or consists of the nucleic acid sequence as recited in SEQ ID NO:1 (encoding the INSP052 A exon 1 polypeptide), SEQ  
20 ID NO:3 (encoding the INSP052 exon 2 polypeptide), SEQ ID NO:5 (encoding the INSP052 exon 3 polypeptide), SEQ ID NO:7 (encoding the INSP052 exon 4 polypeptide), SEQ ID NO:9 (encoding the INSP052 exon 5 polypeptide), SEQ ID NO:11 (encoding the INSP052 exon 6 polypeptide), SEQ ID NO:13 (encoding the INSP052 exon 7 polypeptide), SEQ ID NO:15 (encoding the INSP052 polypeptide), or SEQ ID NO:17  
25 (encoding the INSP055 polypeptide), or is a redundant equivalent or fragment of any one of these sequences.

Combining the sequences recited in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:13 produces the sequence recited in SEQ ID NO:15.

30 In a third aspect, the invention provides a purified nucleic acid molecule which hybridizes

under high stringency conditions with a nucleic acid molecule of the second aspect of the invention.

In a fourth aspect, the invention provides a vector, such as an expression vector, that contains a nucleic acid molecule of the second or third aspect of the invention.

5 In a fifth aspect, the invention provides a host cell transformed with a vector of the fourth aspect of the invention.

In a sixth aspect, the invention provides a ligand which binds specifically to, and which preferably inhibits the activity of a polypeptide of the first aspect of the invention.

By "the activity of a polypeptide of the invention" and similar expressions, we refer to  
10 activity characteristic of immunoglobulin domain-containing cell surface recognition molecules.

In a seventh aspect, the invention provides a compound that is effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

15 A compound of the seventh aspect of the invention may either increase (agonise) or decrease (antagonise) the level of expression of the gene or the activity of the polypeptide.

Importantly, the identification of the function of the INSP052 and INSP055 polypeptides allows for the design of screening methods capable of identifying compounds that are effective in the treatment and/or diagnosis of disease. Ligands and compounds according to  
20 the sixth and seventh aspects of the invention may be identified using such methods. These methods are included as aspects of the present invention.

In an eighth aspect, the invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a ligand of the sixth aspect of the invention,  
25 or a compound of the seventh aspect of the invention, for use in therapy or diagnosis.

These molecules may also be used in the manufacture of a medicament for the treatment of diseases including, but not limited to, cell proliferative disorders, autoimmune/inflammatory disorders, cardiovascular disorders, neurological and psychiatric disorders, developmental disorders, genetic disorders, metabolic disorders,  
30 infections and other pathological conditions. These diseases preferably include neoplasm, cancer, brain tumour, glioma, bone tumor, lung tumor, breast tumour, prostate tumour,

colon tumour, hemangioma, myeloproliferative disorder, leukemia, hematological disease, neutropenia, thrombocytopenia, angiogenesis disorders, dermatological disease, ageing, wounds, burns, fibrosis, cardiovascular disease, restenosis, heart disease, peripheral vascular disease, coronary artery disease, oedema, thromboembolism, dysmenorrhea,

5 endometriosis, pre-eclampsia, lung disease, COPD, asthma, bone disease, renal disease, glomerulonephritis, liver disease, Crohn's disease, gastritis, ulcerative colitis, ulcer, immune disorder, autoimmune disease, arthritis, rheumatoid arthritis, psoriasis, epidermolysis bullosa, systemic lupus erythematosus, ankylosing spondylitis, Lyme disease, multiple sclerosis, neurodegeneration, stroke, brain/spinal cord injury, Alzheimer's

10 disease, Parkinson's disease, motor neurone disease, neuromuscular disease, HIV, AIDS, cytomegalovirus infection, fungal infection, ocular disorder, macular degeneration, glaucoma, diabetic retinopathy, ocular hypertension and other conditions in which immunoglobulin domain containing cell surface recognition molecules are implicated.

In a ninth aspect, the invention provides a method of diagnosing a disease in a patient,

15 comprising assessing the level of expression of a natural gene encoding a polypeptide of the first aspect of the invention or the activity of a polypeptide of the first aspect of the invention in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease. Such a method will preferably be carried out *in vitro*. Similar methods may be used for

20 monitoring the therapeutic treatment of disease in a patient, wherein altering the level of expression or activity of a polypeptide or nucleic acid molecule over the period of time towards a control level is indicative of regression of disease.

A preferred method for detecting polypeptides of the first aspect of the invention comprises the steps of: (a) contacting a ligand, such as an antibody, of the sixth aspect of

25 the invention with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

A number of different methods according to the ninth aspect of the invention exist, as the skilled reader will be aware, such as methods of nucleic acid hybridization with short probes, point mutation analysis, polymerase chain reaction (PCR) amplification and

30 methods using antibodies to detect aberrant protein levels. Similar methods may be used on a short or long term basis to allow therapeutic treatment of a disease to be monitored in a patient. The invention also provides kits that are useful in these methods for diagnosing

disease.

In a tenth aspect, the invention provides for the use of the polypeptides of the first aspect of the invention as immunoglobulin domain-containing cell surface recognition molecules. The invention also provides for the use of a nucleic acid molecule according to the second 5 or third aspects of the invention to express a protein that possesses immunoglobulin domain-containing cell surface recognition molecule activity. The invention also provides a method for effecting immunoglobulin domain-containing cell surface recognition molecule activity, said method utilising a polypeptide of the first aspect of the invention.

In an eleventh aspect, the invention provides a pharmaceutical composition comprising a 10 polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, in conjunction with a pharmaceutically-acceptable carrier.

In a twelfth aspect, the present invention provides a polypeptide of the first aspect of the 15 invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in therapy or diagnosis. These molecules may also be used in the manufacture of a medicament for the treatment of diseases including, but not limited to, cell proliferative disorders, 20 autoimmune/inflammatory disorders, cardiovascular disorders, neurological and psychiatric disorders, developmental disorders, genetic disorders, metabolic disorders, infections and other pathological conditions. These diseases preferably include neoplasm, cancer, brain tumour, glioma, bone tumor, lung tumor, breast tumour, prostate tumour, colon tumour, hemangioma, myeloproliferative disorder, leukemia, hematological disease, 25 neutropenia, thrombocytopenia, angiogenesis disorders, dermatological disease, ageing, wounds, burns, fibrosis, cardiovascular disease, restenosis, heart disease, peripheral vascular disease, coronary artery disease, oedema, thromboembolism, dysmenorrhea, endometriosis, pre-eclampsia, lung disease, COPD, asthma, bone disease, renal disease, glomerulonephritis, liver disease, Crohn's disease, gastritis, ulcerative colitis, ulcer, 30 immune disorder, autoimmune disease, arthritis, rheumatoid arthritis, psoriasis, epidermolysis bullosa, systemic lupus erythematosus, ankylosing spondylitis, Lyme disease, multiple sclerosis, neurodegeneration, stroke, brain/spinal cord injury, Alzheimer's

disease, Parkinson's disease, motor neurone disease, neuromuscular disease, HIV, AIDS, cytomegalovirus infection, fungal infection, ocular disorder, macular degeneration, glaucoma, diabetic retinopathy, ocular hypertension and other conditions in which immunoglobulin domain containing cell recognition molecules are implicated.

- 5 In a thirteenth aspect, the invention provides a method of treating a disease in a patient comprising administering to the patient a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention.
- 10 For diseases in which the expression of a natural gene encoding a polypeptide of the first aspect of the invention, or in which the activity of a polypeptide of the first aspect of the invention, is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an agonist. Conversely, for diseases in which the
- 15 expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an antagonist. Examples of such antagonists include antisense nucleic acid molecules, ribozymes and ligands, such as antibodies.
- 20 In a fourteenth aspect, the invention provides transgenic or knockout non-human animals that have been transformed to express higher, lower or absent levels of a polypeptide of the first aspect of the invention. Such transgenic animals are very useful models for the study of disease and may also be used in screening regimes for the identification of compounds that are effective in the treatment or diagnosis of such a disease.
- 25 It should be appreciated that the scope of protection sought for the polypeptides and nucleic acids of the present invention does not extend to nucleic acids or polypeptides present in their natural source. Rather, the polypeptides and nucleic acids claimed by the present invention may be regarded as being "isolated" or "purified". The terms "isolated" and "purified" as used herein refer to a nucleic acid or polypeptide separated from at least
- 30 one other component (e. g., nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. Thus, for example, a polypeptide contained in a tissue extract would constitute an "isolated" or "purified" polypeptide, as would a polypeptide

synthetically or recombinantly produced. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other component normally present in a solution of the same.

It should be noted that the terms "isolated" and "purified" do not denote the method by which 5 the polypeptide or nucleic acid is obtained or the level of purity of the preparation. Thus, such isolated or purified species may be produced recombinantly, isolated directly from the cell or tissue of interest or produced synthetically based on the determined sequences.

A summary of standard techniques and procedures which may be employed in order to utilise the invention is given below. It will be understood that this invention is not limited 10 to the particular methodology, protocols, cell lines, vectors and reagents described. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and it is not intended that this terminology should limit the scope of the present invention. The extent of the invention is limited only by the terms of the appended claims.

15 Standard abbreviations for nucleotides and amino acids are used in this specification.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.

Such techniques are explained fully in the literature. Examples of particularly suitable texts 20 for consultation include the following: Sambrook Molecular Cloning; A Laboratory Manual, Second Edition (1989); DNA Cloning, Volumes I and II (D.N Glover ed. 1985); Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.I. Freshney ed. 1986); Immobilized Cells and Enzymes 25 (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the Methods in Enzymology series (Academic Press, Inc.), especially volumes 154 & 155; Gene Transfer Vectors for Mammalian Cells (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds. 1987, Academic Press, London); Scopes, (1987) Protein Purification: 30 Principles and Practice, Second Edition (Springer Verlag, N.Y.); and Handbook of Experimental Immunology, Volumes I-IV (D.M. Weir and C. C. Blackwell eds. 1986).

As used herein, the term "polypeptide" includes any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e. peptide isosteres. This term refers both to short chains (peptides and oligopeptides) and to longer chains (proteins).

- 5 The polypeptide of the present invention may be in the form of a mature protein or may be a pre-, pro- or prepro- protein that can be activated by cleavage of the pre-, pro- or prepro-portion to produce an active mature polypeptide. In such polypeptides, the pre-, pro- or prepro- sequence may be a leader or secretory sequence or may be a sequence that is employed for purification of the mature polypeptide sequence.
- 10 The polypeptide of the first aspect of the invention may form part of a fusion protein. For example, it is often advantageous to include one or more additional amino acid sequences which may contain secretory or leader sequences, pro-sequences, sequences which aid in purification, or sequences that confer higher protein stability, for example during recombinant production. Alternatively or additionally, the mature polypeptide may be
- 15 fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol).

Polypeptides may contain amino acids other than the 20 gene-encoded amino acids, modified either by natural processes, such as by post-translational processing or by chemical modification techniques which are well known in the art. Among the known

- 20 modifications which may commonly be present in polypeptides of the present invention are glycosylation, lipid attachment, sulphation, gamma-carboxylation, for instance of glutamic acid residues, hydroxylation and ADP-ribosylation. Other potential modifications include acetylation, acylation, amidation, covalent attachment of flavin, covalent attachment of a haeme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent
- 25 attachment of a lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulphide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, GPI anchor formation, iodination, methylation, myristylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenylation, transfer-RNA mediated addition of amino acids
- 30 to proteins such as arginylation, and ubiquitination.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino

or carboxyl terminus in a polypeptide, or both, by a covalent modification is common in naturally-occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention.

The modifications that occur in a polypeptide often will be a function of how the

5 polypeptide is made. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns vary between different types of host cell.

10 The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally-occurring polypeptides (for example purified from cell culture), recombinantly-produced polypeptides (including fusion proteins), synthetically-produced polypeptides or polypeptides that are produced by a combination of these methods.

15 The functionally-equivalent polypeptides of the first aspect of the invention may be polypeptides that are homologous to the INSP052 and INSP055 polypeptides. Two polypeptides are said to be "homologous", as the term is used herein, if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of the other polypeptide. "Identity" indicates that at any particular position in the aligned

20 sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed.,

25 Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991).

Homologous polypeptides therefore include natural biological variants (for example, allelic

30 variants or geographical variations within the species from which the polypeptides are derived) and mutants (such as mutants containing amino acid substitutions, insertions or deletions) of the INSP052 and INSP055 polypeptides. Such mutants may include

polypeptides in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and 5 Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg; or among the aromatic residues Phe and Tyr. Particularly preferred are variants in which several, i.e. between 5 and 10, 1 and 5, 1 and 3, 1 and 2 or just 1 amino acids are substituted, deleted or added in any combination. Especially preferred are silent substitutions, additions and deletions, which do not alter the properties and activities 10 of the protein. Also especially preferred in this regard are conservative substitutions. Such mutants also include polypeptides in which one or more of the amino acid residues includes a substituent group.

Typically, greater than 30% identity between two polypeptides is considered to be an indication of functional equivalence. Preferably, functionally equivalent polypeptides of 15 the first aspect of the invention have a degree of sequence identity with the INSP052 and INSP055 polypeptides, or with active fragments thereof, of greater than 80%. More preferred polypeptides have degrees of identity of greater than 85%, 90%, 95%, 98% or 99%, respectively.

The functionally-equivalent polypeptides of the first aspect of the invention may also be 20 polypeptides which have been identified using one or more techniques of structural alignment. For example, the Inpharmatica Genome Threader<sup>TM</sup> technology that forms one aspect of the search tools used to generate the Biopendium search database may be used (see co-pending International Patent Application No. PCT/GB01/01105) to identify polypeptides of presently-unknown function which, while having low sequence identity as 25 compared to the INSP052 and INSP055 polypeptides, are predicted to be immunoglobulin domain-containing cell surface recognition molecules, said method utilising a polypeptide of the first aspect of the invention, by virtue of sharing significant structural homology with the INSP052 and INSP055 polypeptide sequences. By "significant structural homology" is meant that the Inpharmatica Genome Threader<sup>TM</sup> predicts two proteins to 30 share structural homology with a certainty of at least 10% and more preferably, at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and above.

The polypeptides of the first aspect of the invention also include fragments of the INSP052

and INSP055 polypeptides and fragments of the functional equivalents of the INSP052 and INSP055 polypeptides, provided that those fragments retain immunoglobulin domain-containing cell surface recognition molecule activity or have an antigenic determinant in common with the INSP052 and INSP055 polypeptides.

- 5 As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of the INSP052 and INSP055 polypeptides or one of its functional equivalents. The fragments should comprise at least n consecutive amino acids from the sequence and, depending on the particular sequence, n preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small  
10 fragments may form an antigenic determinant.

Such fragments may be "free-standing", i.e. not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the fragment of the invention most preferably forms a single continuous region. For instance, certain preferred  
15 embodiments relate to a fragment having a pre - and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger polypeptide.

- 20 The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may be employed to isolate or to identify clones expressing the polypeptides of the invention or to purify the polypeptides by affinity chromatography. The antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent  
25 to the skilled reader.

The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art. As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')2 and Fv, which are capable of binding to the  
30 antigenic determinant in question. Such antibodies thus bind to the polypeptides of the first aspect of the invention.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or

horse, may be immunised with a polypeptide of the first aspect of the invention. The polypeptide used to immunise the animal can be derived by recombinant DNA technology or can be synthesized chemically. If desired, the polypeptide can be conjugated to a carrier protein. Commonly used carriers to which the polypeptides may be chemically coupled

5 include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The coupled polypeptide is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by immunoaffinity chromatography.

Monoclonal antibodies to the polypeptides of the first aspect of the invention can also be

10 readily produced by one skilled in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known (see, for example, Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, 77-96 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985).

15 Panels of monoclonal antibodies produced against the polypeptides of the first aspect of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc. Monoclonal antibodies are particularly useful in purification of the individual polypeptides against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the

20 art, and cloned and expressed in appropriate vectors.

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions (see, for example, Liu *et al.*, *Proc. Natl. Acad. Sci. USA*, 84, 3439 (1987)), may also be of use.

The antibody may be modified to make it less immunogenic in an individual, for example

25 by humanisation (see Jones *et al.*, *Nature*, 321, 522 (1986); Verhoeyen *et al.*, *Science*, 239, 1534 (1988); Kabat *et al.*, *J. Immunol.*, 147, 1709 (1991); Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, 86, 10029 (1989); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA*, 88, 34181 (1991); and Hodgson *et al.*, *Bio/Technology*, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other

30 amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody. The humanised antibody thus closely resembles a human antibody but has the binding

ability of the donor antibody.

In a further alternative, the antibody may be a "bispecific" antibody, that is an antibody having two different antigen binding domains, each domain being directed against a different epitope.

- 5 Phage display technology may be utilised to select genes which encode antibodies with binding activities towards the polypeptides of the invention either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant antibodies, or from naive libraries (McCafferty, J. *et al.*, (1990), *Nature* 348, 552-554; Marks, J. *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can
- 10 also be improved by chain shuffling (Clackson, T. *et al.*, (1991) *Nature* 352, 624-628).

Antibodies generated by the above techniques, whether polyclonal or monoclonal, have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-detectable reagent such as

- 15 a radioisotope, a fluorescent molecule or an enzyme.

Preferred nucleic acid molecules of the second and third aspects of the invention are those which encode the polypeptide sequences recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and/or SEQ ID NO:16, or SEQ ID NO:18, and functionally equivalent polypeptides. These nucleic acid

- 20 molecules may be used in the methods and applications described herein. The nucleic acid molecules of the invention preferably comprise at least n consecutive nucleotides from the sequences disclosed herein where, depending on the particular sequence, n is 10 or more (for example, 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

The nucleic acid molecules of the invention also include sequences that are complementary

- 25 to nucleic acid molecules described above (for example, for antisense or probing purposes).

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance cDNA, synthetic DNA or genomic DNA. Such nucleic acid molecules may be obtained by cloning, by chemical synthetic

- 30 techniques or by a combination thereof. The nucleic acid molecules can be prepared, for example, by chemical synthesis using techniques such as solid phase phosphoramidite chemical synthesis, from genomic or cDNA libraries or by separation from an organism.

RNA molecules may generally be generated by the *in vitro* or *in vivo* transcription of DNA sequences.

The nucleic acid molecules may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The term "nucleic acid molecule" also includes analogues of DNA and RNA, such as those containing modified backbones, and peptide nucleic acids (PNA). The term "PNA", as used herein, refers to an antisense molecule or an anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, which preferably ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in a cell, where they preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) *Anticancer Drug Des.* 8:53-63).

These molecules also may have a different sequence which, as a result of the degeneracy of the genetic code, encode a polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and/or SEQ ID NO:16, or SEQ ID NO:18. Such molecules may include, but are not limited to, the coding sequence for the mature polypeptide by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pro-, pre- or prepro- polypeptide sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with further additional, non-coding sequences, including non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals), ribosome binding and mRNA stability. The nucleic acid molecules may also include additional sequences which encode additional amino acids, such as those which provide additional functionalities.

The nucleic acid molecules of the second and third aspects of the invention may also encode the fragments or the functional equivalents of the polypeptides and fragments of the first aspect of the invention. Such a nucleic acid molecule may be a naturally-occurring variant such as a naturally-occurring allelic variant, or the molecule may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid

molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or insertions. The substitutions, deletions or insertions may involve one or more nucleotides. The variants may be altered in coding

5 or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or insertions.

The nucleic acid molecules of the invention can also be engineered, using methods generally known in the art, for a variety of reasons, including modifying the cloning, processing, and/or expression of the gene product (the polypeptide). DNA shuffling by

10 random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides are included as techniques which may be used to engineer the nucleotide sequences. Site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations and so forth.

15 Nucleic acid molecules which encode a polypeptide of the first aspect of the invention may be ligated to a heterologous sequence so that the combined nucleic acid molecule encodes a fusion protein. Such combined nucleic acid molecules are included within the second or third aspects of the invention. For example, to screen peptide libraries for inhibitors of the activity of the polypeptide, it may be useful to express, using such a combined nucleic acid  
20 molecule, a fusion protein that can be recognised by a commercially-available antibody. A fusion protein may also be engineered to contain a cleavage site located between the sequence of the polypeptide of the invention and the sequence of a heterologous protein so that the polypeptide may be cleaved and purified away from the heterologous protein.

The nucleic acid molecules of the invention also include antisense molecules that are

25 partially complementary to nucleic acid molecules encoding polypeptides of the present invention and that therefore hybridize to the encoding nucleic acid molecules (hybridization). Such antisense molecules, such as oligonucleotides, can be designed to recognise, specifically bind to and prevent transcription of a target nucleic acid encoding a polypeptide of the invention, as will be known by those of ordinary skill in the art (see, for  
30 example, Cohen, J.S., Trends in Pharm. Sci., 10, 435 (1989), Okano, J. Neurochem. 56, 560 (1991); O'Connor, J. Neurochem 56, 560 (1991); Lee *et al.*, Nucleic Acids Res 6, 3073 (1979); Cooney *et al.*, Science 241, 456 (1988); Dervan *et al.*, Science 251, 1360 (1991).

The term "hybridization" as used herein refers to the association of two nucleic acid molecules with one another by hydrogen bonding. Typically, one molecule will be fixed to a solid support and the other will be free in solution. Then, the two molecules may be placed in contact with one another under conditions that favour hydrogen bonding. Factors 5 that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase molecule to the solid support (Denhardt's reagent or BLOTO); the concentration of the molecules; use of compounds to increase the rate of association of molecules (dextran sulphate or polyethylene glycol); and the stringency of the washing conditions following 10 hybridization (see Sambrook *et al.* [*supra*]).

The inhibition of hybridization of a completely complementary molecule to a target molecule may be examined using a hybridization assay, as known in the art (see, for example, Sambrook *et al* [*supra*]). A substantially homologous molecule will then compete for and inhibit the binding of a completely homologous molecule to the target molecule 15 under various conditions of stringency, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

"Stringency" refers to conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. High stringency 20 hybridisation conditions are defined as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65°C. Low stringency conditions involve the hybridisation 25 reaction being carried out at 35°C (see Sambrook *et al.* [*supra*]). Preferably, the conditions used for hybridization are those of high stringency.

Preferred embodiments of this aspect of the invention are nucleic acid molecules that are at least 70% identical over their entire length to a nucleic acid molecule encoding the INSP052 or INSP055 polypeptides (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID 30 NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and/or SEQ ID NO:15, or SEQ ID NO:17) and nucleic acid molecules that are substantially complementary to such nucleic acid molecules. Preferably, a nucleic acid molecule according to this aspect of the

invention comprises a region that is at least 80% identical over its entire length to the coding sequence for SEQ ID NO:2 given in SEQ ID NO:1, the coding sequence for SEQ ID NO:4 given in SEQ ID NO:3, the coding sequences for SEQ ID NO:6 given in SEQ ID NO:5, the coding sequence for SEQ ID NO:8 given in SEQ ID NO:7, the coding sequence 5 for SEQ ID NO:10 given in SEQ ID NO:9, the coding sequence for SEQ ID NO:12 given in SEQ ID NO:11, the coding sequence for SEQ ID NO:14 given in SEQ ID NO:13, the coding sequence for SEQ ID NO:16 given in SEQ ID NO:15, the coding sequence for SEQ ID NO:18 given in SEQ ID NO:17 or is a nucleic acid molecule that is complementary thereto. In this regard, nucleic acid molecules at least 90%, preferably at least 95%, more 10 preferably at least 98% or 99% identical over their entire length to the same are particularly preferred. Preferred embodiments in this respect are nucleic acid molecules that encode polypeptides which retain substantially the same biological function or activity 15 as the INSP052 and INSP055 polypeptides.

The invention also provides a process for detecting a nucleic acid molecule of the 15 invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting any such duplexes that are formed.

As discussed additionally below in connection with assays that may be utilised according to the invention, a nucleic acid molecule as described above may be used as a hybridization 20 probe for RNA, cDNA or genomic DNA, in order to isolate full-length cDNAs and genomic clones encoding the INSP052 and INSP055 polypeptides and to isolate cDNA and genomic clones of homologous or orthologous genes that have a high sequence similarity to the gene encoding this polypeptide.

In this regard, the following techniques, among others known in the art, may be utilised 25 and are discussed below for purposes of illustration. Methods for DNA sequencing and analysis are well known and are generally available in the art and may, indeed, be used to practice many of the embodiments of the invention discussed herein. Such methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 30 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proof-reading exonucleases such as those found in the ELONGASE Amplification System marketed by Gibco/BRL (Gaithersburg, MD). Preferably, the sequencing process may be automated

using machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), the Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

One method for isolating a nucleic acid molecule encoding a polypeptide with an equivalent function to that of the INSP052 and INSP055 polypeptides is to probe a genomic or cDNA library with a natural or artificially-designed probe using standard procedures that are recognised in the art (see, for example, "Current Protocols in Molecular Biology", Ausubel *et al.* (eds). Greene Publishing Association and John Wiley Interscience, New York, 1989,1992). Probes comprising at least 15, preferably at least 30, and more preferably at least 50, contiguous bases that correspond to, or are complementary to, nucleic acid sequences from the appropriate encoding gene (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17) are particularly useful probes. Such probes may be labelled with an analytically-detectable reagent to facilitate their identification. Useful reagents include, but are not limited to, radioisotopes, fluorescent dyes and enzymes that are capable of catalysing the formation of a detectable product. Using these probes, the ordinarily skilled artisan will be capable of isolating complementary copies of genomic DNA, cDNA or RNA polynucleotides encoding proteins of interest from human, mammalian or other animal sources and screening such sources for related sequences, for example, for additional members of the family, type and/or subtype.

In many cases, isolated cDNA sequences will be incomplete, in that the region encoding the polypeptide will be cut short, normally at the 5' end. Several methods are available to obtain full length cDNAs, or to extend short cDNAs. Such sequences may be extended utilising a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed is based on the method of Rapid Amplification of cDNA Ends (RACE; see, for example, Frohman *et al.*, PNAS USA 85, 8998-9002, 1988). Recent modifications of this technique, exemplified by the MarathonTM technology (Clontech Laboratories Inc.), for example, have significantly simplified the search for longer cDNAs. A slightly different technique, termed "restriction-site" PCR, uses universal primers to retrieve unknown nucleic acid sequence adjacent a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). Inverse PCR may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.* (1988)

Nucleic Acids Res. 16:8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.* (1991) PCR Methods Applic., 1, 111-119). Another method which may be used to retrieve unknown sequences is that of

5 Parker, J.D. *et al.* (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinderTM libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-  
10 selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

15 In one embodiment of the invention, the nucleic acid molecules of the present invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important step in the confirmatory correlation of those sequences  
20 with the gene-associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch Medical Library). The relationships between genes and diseases that have been mapped to  
25 the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular genomic region, any sequences mapping to that area may represent associated or  
30 regulatory genes for further investigation. The nucleic acid molecule may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

The nucleic acid molecules of the present invention are also valuable for tissue localisation. Such techniques allow the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques include *in situ* hybridization techniques and nucleotide amplification techniques, such as

- 5 PCR. Results from these studies provide an indication of the normal functions of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable insights into the role of mutant polypeptides in disease. Such inappropriate expression may be of a temporal, spatial or quantitative nature.
- 10 The vectors of the present invention comprise nucleic acid molecules of the invention and may be cloning or expression vectors. The host cells of the invention, which may be transformed, transfected or transduced with the vectors of the invention may be prokaryotic or eukaryotic.

The polypeptides of the invention may be prepared in recombinant form by expression of

- 15 their encoding nucleic acid molecules in vectors contained within a host cell. Such expression methods are well known to those of skill in the art and many are described in detail by Sambrook *et al* (*supra*) and Fernandez & Hoeffler (1998, eds. "Gene expression systems. Using nature for the art of expression". Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto).

- 20 Generally, any system or vector that is suitable to maintain, propagate or express nucleic acid molecules to produce a polypeptide in the required host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those described in Sambrook *et al.*, (*supra*). Generally, the encoding gene can be placed under the control of a control element
- 25 such as a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator, so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the transformed host cell.

Examples of suitable expression systems include, for example, chromosomal, episomal and virus-derived systems, including, for example, vectors derived from: bacterial plasmids,

- 30 bacteriophage, transposons, yeast episomes, insertion elements, yeast chromosomal elements, viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, or combinations

thereof, such as those derived from plasmid and bacteriophage genetic elements, including cosmids and phagemids. Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid.

- Particularly suitable expression systems include microorganisms such as bacteria
- 5 transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (for example, baculovirus); plant cell systems transformed with virus expression vectors (for example, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (for example, Ti or pBR322 plasmids); or
- 10 animal cell systems. Cell-free translation systems can also be employed to produce the polypeptides of the invention.

Introduction of nucleic acid molecules encoding a polypeptide of the present invention into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, *Basic Methods in Molecular Biology* (1986) and Sambrook *et al.*, [supra].

- 15 Particularly suitable methods include calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection; electroporation, transduction, scrape loading, ballistic introduction or infection (see Sambrook *et al.*, 1989 [supra]; Ausubel *et al.*, 1991 [supra]; Spector, Goldman & Leinwald, 1998). In eukaryotic cells, expression systems may either be transient (for
- 20 example, episomal) or permanent (chromosomal integration) according to the needs of the system.

The encoding nucleic acid molecule may or may not include a sequence encoding a control sequence, such as a signal peptide or leader sequence, as desired, for example, for secretion of the translated polypeptide into the lumen of the endoplasmic reticulum, into the

- 25 periplasmic space or into the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals. Leader sequences can be removed by the bacterial host in post-translational processing.

In addition to control sequences, it may be desirable to add regulatory sequences that allow for regulation of the expression of the polypeptide relative to the growth of the host cell.

- 30 Examples of regulatory sequences are those which cause the expression of a gene to be increased or decreased in response to a chemical or physical stimulus, including the presence of a regulatory compound or to various temperature or metabolic conditions.

Regulatory sequences are those non-translated regions of the vector, such as enhancers, promoters and 5' and 3' untranslated regions. These interact with host cellular proteins to carry out transcription and translation. Such regulatory sequences may vary in their strength and specificity. Depending on the vector system and host utilised, any number of 5 suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript phagemid (Stratagene, LaJolla, CA) or pSport<sup>TM</sup> plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the 10 genomes of plant cells (for example, heat shock, RUBISCO and storage protein genes) or from plant viruses (for example, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence, vectors based on SV40 or EBV may be used with an 15 appropriate selectable marker.

An expression vector is constructed so that the particular nucleic acid coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the regulatory sequences being such that the coding sequence is transcribed under the "control" of the regulatory sequences, i.e., 20 RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence. In some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame.

The control sequences and other regulatory sequences may be ligated to the nucleic acid 25 coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector that already contains the control sequences and an appropriate restriction site.

For long-term, high-yield production of a recombinant polypeptide, stable expression is preferred. For example, cell lines which stably express the polypeptide of interest may be 30 transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days

in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to

5 the cell type.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalised cell lines available from the American Type Culture Collection (ATCC) including, but not limited to, Chinese hamster ovary (CHO), HeLa, baby hamster kidney (BHK), monkey kidney (COS), C127, 3T3, BHK, HEK 293, Bowes melanoma and

10 human hepatocellular carcinoma (for example Hep G2) cells and a number of other cell lines.

In the baculovirus system, the materials for baculovirus/insect cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego CA (the "MaxBac" kit). These techniques are generally known to those skilled in the art and are

15 described fully in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Particularly suitable host cells for use in this system include insect cells such as Drosophila S2 and Spodoptera Sf9 cells.

There are many plant cell culture and whole plant genetic expression systems known in the art. Examples of suitable plant cellular genetic expression systems include those described

20 in US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, Phytochemistry 30, 3861-3863 (1991).

In particular, all plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be utilised, so that whole plants are recovered which contain the

25 transferred gene. Practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugar cane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Examples of particularly preferred bacterial host cells include streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells.

30 Examples of particularly suitable host cells for fungal expression include yeast cells (for example, *S. cerevisiae*) and *Aspergillus* cells.

Any number of selection systems are known in the art that may be used to recover transformed cell lines. Examples include the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1980) *Cell* 22:817-23) genes that can be employed in tk- or aprt $\pm$  cells, respectively.

- 5 Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dihydrofolate reductase (DHFR) that confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al* (1981) *J. Mol. Biol.* 150:1-14) and als or pat, which confer resistance to chlorsulfuron and
- 10 10 phosphinotricin acetyltransferase, respectively. Additional selectable genes have been described, examples of which will be clear to those of skill in the art.

Although the presence or absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the relevant sequence is inserted within a marker gene sequence, transformed 15 cells containing the appropriate sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

- 20 Alternatively, host cells that contain a nucleic acid sequence encoding a polypeptide of the invention and which express said polypeptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassays, for example, fluorescence activated cell sorting (FACS) or immunoassay techniques (such as the enzyme-linked
- 25 25 immunosorbent assay [ELISA] and radioimmunoassay [RIA]), that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein (see Hampton, R. *et al.* (1990) *Serological Methods, a Laboratory Manual*, APS Press, St Paul, MN) and Maddox, D.E. *et al.* (1983) *J. Exp. Med.* 158, 1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art 30 and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting sequences related to nucleic acid molecules encoding polypeptides of the present invention include oligolabelling, nick

translation, end-labelling or PCR amplification using a labelled polynucleotide. Alternatively, the sequences encoding the polypeptide of the invention may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of 5 an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include 10 radionuclides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells, 15 or by germ line therapy to incorporate heritable modifications. Such transgenic animals may be particularly useful in the generation of animal models for drug molecules effective as modulators of the polypeptides of the present invention.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, 20 anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography is particularly useful for purification. Well known techniques for refolding proteins may be employed to regenerate an active conformation when the polypeptide is denatured during 25 isolation and or purification.

Specialised vector constructions may also be used to facilitate purification of proteins, as desired, by joining sequences encoding the polypeptides of the invention to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Examples of such purification-facilitating domains include metal chelating 30 peptides such as histidine-tryptophan modules that allow purification on immobilised metals, protein A domains that allow purification on immobilised immunoglobulin, and the domain utilised in the FLAGS extension/affinity purification system (Immunex Corp.,

Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the polypeptide of the invention may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing the polypeptide of the invention fused to several histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilised metal ion affinity chromatography as described in Porath, J. *et al.* (1992), *Prot. Exp. Purif.* 3: 263-281) while the thioredoxin or enterokinase cleavage site provides a means for purifying the polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. *et al.* (1993; *DNA Cell Biol.* 12:441-453).

If the polypeptide is to be expressed for use in screening assays, generally it is preferred that it be produced at the surface of the host cell in which it is expressed. In this event, the host cells may be harvested prior to use in the screening assay, for example using techniques such as fluorescence activated cell sorting (FACS) or immunoaffinity 15. techniques. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the expressed polypeptide. If polypeptide is produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

The polypeptide of the invention can be used to screen libraries of compounds in any of a variety of drug screening techniques. Such compounds may activate (agonise) or inhibit 20 (antagonise) the level of expression of the gene or the activity of the polypeptide of the invention and form a further aspect of the present invention. Preferred compounds are effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

25 Agonist or antagonist compounds may be isolated from, for example, cells, cell-free preparations, chemical libraries or natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors or structural or functional mimetics. For a suitable review of such screening techniques, see Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

30 Compounds that are most likely to be good antagonists are molecules that bind to the polypeptide of the invention without inducing the biological effects of the polypeptide upon binding to it. Potential antagonists include small organic molecules, peptides,

polypeptides and antibodies that bind to the polypeptide of the invention and thereby inhibit or extinguish its activity. In this fashion, binding of the polypeptide to normal cellular binding molecules may be inhibited, such that the normal biological activity of the polypeptide is prevented.

- 5 The polypeptide of the invention that is employed in such a screening technique may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. In general, such screening procedures may involve using appropriate cells or cell membranes that express the polypeptide that are contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The functional response of
- 10 10 the cells contacted with the test compound is then compared with control cells that were not contacted with the test compound. Such an assay may assess whether the test compound results in a signal generated by activation of the polypeptide, using an appropriate detection system. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist in the presence of the test
- 15 15 compound is observed.

A preferred method for identifying an agonist or antagonist compound of a polypeptide of the present invention comprises:

- (a) contacting a cell expressing on the surface thereof the polypeptide according to the first aspect of the invention, the polypeptide being associated with a second component capable 20 of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and
- (b) determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the 25 polypeptide.

A further preferred method for identifying an agonist or antagonist of a polypeptide of the invention comprises:

- (a) contacting a cell expressing on the surface thereof the polypeptide, the polypeptide being associated with a second component capable of providing a detectable signal in 30 response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

(b) determining whether the compound binds to and activates or inhibits the polypeptide by comparing the level of a signal generated from the interaction of the compound with the polypeptide with the level of a signal in the absence of the compound.

In further preferred embodiments, the general methods that are described above may

5 further comprise conducting the identification of agonist or antagonist in the presence of labelled or unlabelled ligand for the polypeptide.

In another embodiment of the method for identifying agonist or antagonist of a polypeptide of the present invention comprises:

determining the inhibition of binding of a ligand to cells which have a polypeptide of the  
10 invention on the surface thereof, or to cell membranes containing such a polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and determining the amount of ligand bound to the polypeptide. A compound capable of causing reduction of binding of a ligand is considered to be an agonist or antagonist. Preferably the ligand is labelled.

15 More particularly, a method of screening for a polypeptide antagonist or agonist compound comprises the steps of:

(a) incubating a labelled ligand with a whole cell expressing a polypeptide according to the invention on the cell surface, or a cell membrane containing a polypeptide of the invention,

(b) measuring the amount of labelled ligand bound to the whole cell or the cell membrane;

20 (c) adding a candidate compound to a mixture of labelled ligand and the whole cell or the cell membrane of step (a) and allowing the mixture to attain equilibrium;

(d) measuring the amount of labelled ligand bound to the whole cell or the cell membrane after step (c); and

(e) comparing the difference in the labelled ligand bound in step (b) and (d), such that the  
25 compound which causes the reduction in binding in step (d) is considered to be an agonist or antagonist.

In certain of the embodiments described above, simple binding assays may be used, in which the adherence of a test compound to a surface bearing the polypeptide is detected by means of a label directly or indirectly associated with the test compound or in an assay

30 involving competition with a labelled competitor. In another embodiment, competitive

drug screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide.

- 5 Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be used to search for compounds that may inhibit or enhance the production of the
- 10 polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different

- 15 small test compounds are synthesised on a solid substrate, which may then be reacted with the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

- 20 The polypeptide of the invention may be used to identify membrane-bound or soluble receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that facilitates its detection or purification, and incubated with a source of the putative receptor
- 25 (for example, a composition of cells, cell membranes, cell supernatants, tissue extracts, or bodily fluids). The efficacy of binding may be measured using biophysical techniques such as surface plasmon resonance and spectroscopy. Binding assays may be used for the purification and cloning of the receptor, but may also identify agonists and antagonists of the polypeptide, that compete with the binding of the polypeptide to its receptor. Standard
- 30 methods for conducting screening assays are well understood in the art.

The invention also includes a screening kit useful in the methods for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, that are described above.

The invention includes the agonists, antagonists, ligands, receptors, substrates and enzymes, and other compounds which modulate the activity or antigenicity of the polypeptide of the invention discovered by the methods that are described above.

The invention also provides pharmaceutical compositions comprising a polypeptide, 5 nucleic acid, ligand or compound of the invention in combination with a suitable pharmaceutical carrier. These compositions may be suitable as therapeutic or diagnostic reagents, as vaccines, or as other immunogenic compositions, as outlined in detail below.

According to the terminology used herein, a composition containing a polypeptide, nucleic acid, ligand or compound [X] is "substantially free of" impurities [herein, Y] when at least 10 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95%, 98% or even 99% by weight.

The pharmaceutical compositions should preferably comprise a therapeutically effective amount of the polypeptide, nucleic acid molecule, ligand, or compound of the invention. 15 The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate, or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, for example, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The 20 animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, 25 time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg. Compositions may be administered individually to a patient or may be administered in combination with other 30 agents, drugs or hormones.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier, for administration of a therapeutic agent. Such carriers include antibodies and other

polypeptides, genes and other therapeutic agents such as liposomes, provided that the carrier does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity.

Suitable carriers may be large, slowly metabolised macromolecules such as proteins,

5 polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulphates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough

10 discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in 15 such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be 20 treated.

The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal or transcutaneous applications (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, 25 enteral, topical, sublingual, intravaginal or rectal means. Gene guns or hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

30 Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion.

Dosage treatment may be a single dose schedule or a multiple dose schedule.

If the activity of the polypeptide of the invention is in excess in a particular disease state, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as described above, along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the polypeptide, such as by blocking the binding of ligands, substrates, enzymes, receptors, or by inhibiting a second signal, and thereby alleviating the abnormal condition. Preferably, such antagonists are antibodies. Most preferably, such antibodies are chimeric and/or humanised to minimise their immunogenicity, as described previously.

10 In another approach, soluble forms of the polypeptide that retain binding affinity for the ligand, substrate, enzyme, receptor, in question, may be administered. Typically, the polypeptide may be administered in the form of fragments that retain the relevant portions. In an alternative approach, expression of the gene encoding the polypeptide can be inhibited using expression blocking techniques, such as the use of antisense nucleic acid molecules (as described above), either internally generated or separately administered. 15 Modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions (signal sequence, promoters, enhancers and introns) of the gene encoding the polypeptide. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple 20 helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. *et al.* (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense 25 molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Such oligonucleotides may be administered or may be generated *in situ* from expression *in vivo*.

In addition, expression of the polypeptide of the invention may be prevented by using ribozymes specific to its encoding mRNA sequence. Ribozymes are catalytically active 30 RNAs that can be natural or synthetic (see for example Usman, N, *et al.*, Curr. Opin. Struct. Biol (1996) 6(4), 527-33). Synthetic ribozymes can be designed to specifically cleave mRNAs at selected positions thereby preventing translation of the mRNAs into

functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesised with non-natural backbones, for example, 2'-O-methyl RNA, to provide protection from ribonuclease degradation and may contain modified 5 bases.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent 10 in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine and butosine, as well as acetyl-, methyl-, thio- and similarly modified forms of adenine, cytidine, guanine, thymine and uridine which are not as easily recognised by endogenous endonucleases.

For treating abnormal conditions related to an under-expression of the polypeptide of the 15 invention and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound that activates the polypeptide, i.e., an agonist as described above, to alleviate the abnormal condition. Alternatively, a therapeutic amount of the polypeptide in combination with a suitable pharmaceutical carrier may be administered to restore the relevant physiological balance of 20 polypeptide.

Gene therapy may be employed to effect the endogenous production of the polypeptide by the relevant cells in the subject. Gene therapy is used to treat permanently the inappropriate production of the polypeptide by replacing a defective gene with a corrected therapeutic gene.

25 Gene therapy of the present invention can occur *in vivo* or *ex vivo*. *Ex vivo* gene therapy requires the isolation and purification of patient cells, the introduction of a therapeutic gene and introduction of the genetically altered cells back into the patient. In contrast, *in vivo* gene therapy does not require isolation and purification of a patient's cells.

The therapeutic gene is typically "packaged" for administration to a patient. Gene delivery 30 vehicles may be non-viral, such as liposomes, or replication-deficient viruses, such as adenovirus as described by Berkner, K.L., in Curr. Top. Microbiol. Immunol., 158, 39-66 (1992) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr.

Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Patent No. 5,252,479. For example, a nucleic acid molecule encoding a polypeptide of the invention may be engineered for expression in a replication-defective retroviral vector. This expression construct may then be isolated and introduced into a packaging cell transduced with a 5 retroviral plasmid vector containing RNA encoding the polypeptide, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo (see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular 10 Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

In situations in which the polypeptides or nucleic acid molecules of the invention are disease-causing agents, the invention provides that they can be used in vaccines to raise 15 antibodies against the disease causing agent.

Vaccines according to the invention may either be prophylactic (ie. to prevent infection) or therapeutic (ie. to treat disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above, which include 20 any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, H. pylori, and other pathogens.

25 Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the 30 blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

The vaccine formulations of the invention may be presented in unit-dose or multi-dose

containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

- 5 This invention also relates to the use of nucleic acid molecules according to the present invention as diagnostic reagents. Detection of a mutated form of the gene characterised by the nucleic acid molecules of the invention which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered
- 10 spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acid molecules for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, ligase chain 15 reaction (LCR), strand displacement amplification (SDA), or other amplification techniques (see Saiki *et al.*, *Nature*, 324, 163-166 (1986); Bej, *et al.*, *Crit. Rev. Biochem. Molec. Biol.*, 26, 301-334 (1991); Birkenmeyer *et al.*, *J. Virol. Meth.*, 35, 117-126 (1991); Van Brunt, *J. Bio/Technology*, 8, 291-294 (1990)) prior to analysis.

In one embodiment, this aspect of the invention provides a method of diagnosing a disease 20 in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to the invention and comparing said level of expression to a control level, wherein a level that is different to said control level is indicative of disease. The method may comprise the steps of:

- a)contacting a sample of tissue from the patient with a nucleic acid probe under stringent 25 conditions that allow the formation of a hybrid complex between a nucleic acid molecule of the invention and the probe;

- b)contacting a control sample with said probe under the same conditions used in step a);

- c)and detecting the presence of hybrid complexes in said samples;

wherein detection of levels of the hybrid complex in the patient sample that differ from 30 levels of the hybrid complex in the control sample is indicative of disease.

A further aspect of the invention comprises a diagnostic method comprising the steps of:

- a)obtaining a tissue sample from a patient being tested for disease;
- b)isolating a nucleic acid molecule according to the invention from said tissue sample; and
- c)diagnosing the patient for disease by detecting the presence of a mutation in the nucleic acid molecule which is associated with disease.

5 To aid the detection of nucleic acid molecules in the above-described methods, an amplification step, for example using PCR, may be included.

Deletions and insertions can be detected by a change in the size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labelled RNA of the invention or alternatively, labelled antisense DNA

10 sequences of the invention. Perfectly-matched sequences can be distinguished from mismatched duplexes by RNase digestion or by assessing differences in melting temperatures. The presence or absence of the mutation in the patient may be detected by contacting DNA with a nucleic acid probe that hybridises to the DNA under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded

15 molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation in the corresponding portion of the DNA strand.

Such diagnostics are particularly useful for prenatal and even neonatal testing.

20 Point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by other well-known techniques, such as direct DNA sequencing or single-strand conformational polymorphism, (see Orita *et al.*, Genomics, 5, 874-879 (1989)). For example, a sequencing primer may be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence

25 determination is performed by conventional procedures with radiolabelled nucleotides or by automatic sequencing procedures with fluorescent-tags. Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. Further, point mutations and other sequence variations, such as polymorphisms, can be detected as described above, for example,

30 through the use of allele-specific oligonucleotides for PCR amplification of sequences that differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, *Science* (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and

5 S1 protection or the chemical cleavage method (see Cotton *et al.*, *Proc. Natl. Acad. Sci. USA* (1985) 85: 4397-4401).

In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by *in situ* analysis (see, for example, Keller *et al.*, *DNA Probes*, 2nd Ed., Stockton Press, New York,

10 10 N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. Fluorescence *in situ* hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, *Science*, 250, 559-562 (1990), and Trask *et al.*, *Trends, Genet.*, 7, 149-154 (1991)).

15 15 In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic

20 20 variability (see for example: M.Chee *et al.*, *Science* (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in PCT application WO95/11995 (Chee *et al*); Lockhart, D. J. *et al.* (1996) *Nat. Biotech.* 14: 1675-1680); and Schena, M. *et al.* (1996) *Proc. Natl. Acad. Sci.* 93: 10614-10619. Oligonucleotide pairs may range from two to over one million. The oligomers are

25 25 synthesized at designated areas on a substrate using a light-directed chemical process. The substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/251116 (Baldeschweiler *et*

30 30 *al*). In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such

as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus), materials (any suitable solid support), and machines (including robotic instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and over one million which lends itself to the efficient use  
5 of commercially-available instrumentation.

In addition to the methods discussed above, diseases may be diagnosed by methods comprising determining, from a sample derived from a subject, an abnormally decreased or increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the  
10 quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

Assay techniques that can be used to determine levels of a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art and are  
15 discussed in some detail above (including radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays). This aspect of the invention provides a diagnostic method which comprises the steps of: (a) contacting a ligand as described above with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

20 Protocols such as ELISA, RIA, and FACS for measuring polypeptide levels may additionally provide a basis for diagnosing altered or abnormal levels of polypeptide expression. Normal or standard values for polypeptide expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably humans, with antibody to the polypeptide under conditions suitable for complex formation  
25 The amount of standard complex formation may be quantified by various methods, such as by photometric means.

Antibodies which specifically bind to a polypeptide of the invention may be used for the diagnosis of conditions or diseases characterised by expression of the polypeptide, or in assays to monitor patients being treated with the polypeptides, nucleic acid molecules,  
30 ligands and other compounds of the invention. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for the polypeptide include methods that utilise the antibody and a label to detect

the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

- 5 Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may
- 10 also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

A diagnostic kit of the present invention may comprise:

- (a) a nucleic acid molecule of the present invention;
- (b) a polypeptide of the present invention; or
- 15 (c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the

20 diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic acid molecules, at least one of which may be a nucleic acid molecule according to the invention.

- 25 To detect polypeptide according to the invention, a diagnostic kit may comprise one or more antibodies that bind to a polypeptide according to the invention; and a reagent useful for the detection of a binding reaction between the antibody and the polypeptide.

Such kits will be of use in diagnosing a disease or susceptibility to disease, including, but not limited to, diseases including, but not limited to, cell proliferative disorders, 30 autoimmune/inflammatory disorders, cardiovascular disorders, neurological and psychiatric disorders, developmental disorders, genetic disorders, metabolic disorders,

infections and other pathological conditions. These diseases preferably include neoplasm, cancer, brain tumour, glioma, bone tumor, lung tumor, breast tumour, prostate tumour, colon tumour, hemangioma, myeloproliferative disorder, leukemia, hematological disease, neutropenia, thrombocytopenia, angiogenesis disorders, dermatological disease, ageing, 5 wounds, burns, fibrosis, cardiovascular disease, restenosis, heart disease, peripheral vascular disease, coronary artery disease, oedema, thromboembolism, dysmenorrhea, endometriosis, pre-eclampsia, lung disease, COPD, asthma bone disease, renal disease, glomerulonephritis, liver disease, Crohn's disease, gastritis, ulcerative colitis, ulcer, immune disorder, autoimmune disease, arthritis, rheumatoid arthritis, psoriasis, 10 epidermolysis bullosa, systemic lupus erythematosus, ankylosing spondylitis, Lyme disease, multiple sclerosis, neurodegeneration, stroke, brain/spinal cord injury, Alzheimer's disease, Parkinson's disease, motor neurone disease, neuromuscular disease, HIV, AIDS, cytomegalovirus infection, fungal infection, ocular disorder, macular degeneration, glaucoma, diabetic retinopathy, ocular hypertension and other conditions in which 15 immunoglobulin domain containing cell recognition molecules are implicated.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to the INSP052 and INSP055 polypeptides.

It will be appreciated that modification of detail may be made without departing from the 20 scope of the invention.

#### **Brief description of the Figures**

**Figure 1:** Results from BLAST against NCBI non-redundant database using full-length INSP052 polypeptide sequence.

25 **Figure 2:** Alignment generated by BLAST between the full-length INSP052 polypeptide sequence and the closest related sequence, biliary glycoprotein H (mouse).

**Figure 3:** Results from BLAST against NCBI non-redundant database using full-length INSP055 polypeptide sequence.

30 **Figure 4:** Alignment generated by BLAST between the full-length INSP055 polypeptide sequence and the closest related sequence, biliary glycoprotein H (mouse).

## Examples

### Example 1 INSP052 and INSP055

The polypeptide sequence derived from combining SEQ ID NO:2, SEQ ID NO:4, SEQ ID 5 NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 and SEQ ID NO:16 which represents the translation of consecutive exons from INSP052 is derived from human genomic sequence. The polynucleotide and polypeptide sequences SEQ ID NO 17 and SEQ ID 18 representing INSP055 are polynucleotide and polypeptide sequences of the mouse orthologue of INSP052 respectively. The existence of a mouse orthologue supports the gene 10 model for the human sequence INSP052.

INSP052 and INSP055 polypeptide sequences represented by SEQ ID NO 16 and SEQ ID NO 18, respectively, are predicted to contain signal peptide sequences and a transmembrane spanning domain.

The polypeptide sequence derived from combining SEQ ID NO:2, SEQ ID NO:4, SEQ ID 15 NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:14 and SEQ ID NO:16 which represents the translation of consecutive exons from INSP052, was used as a BLAST query against the NCBI non-redundant Sequence database. The top ten matches are shown in Figure 1, all of which are immunoglobulin domain containing proteins.

Figure 2 shows the alignment of the INSP052 query sequence to the sequence of the highest 20 matching known protein, biliary glycoprotein H (mouse).

The polypeptide sequence INSP055, was used as a BLAST query against the NCBI non-redundant Sequence database. The top ten matches are shown in Figure 3. Figure 4 shows the alignment of the INSP055 query sequence to the sequence of the highest matching known protein, biliary glycoprotein H (mouse).

25 Expressed sequence tags (ESTs) representing the INSP052 and INSP055 transcripts in human and mouse originate from the following cDNA libraries: brain, including cerebellum, cortex, hippocampus, hypothalamus, medulla oblongata; inner ear and breast. Transcripts are also represented by ESTs from oligodendrogloma, glioblastoma and multiple sclerosis lesions. This suggests that INSP052 can be cloned from the above tissues 30 and may be associated with diseases of the above tissues. Accordingly, the polypeptides,

antibodies and other moieties described herein may have utility in the treating a disease in one of the above tissues.

## Sequence Listing

Note: for amino acids encoded by exon-exon junctions, the amino acid will be assigned to the more 5' exon.

SEQ ID NO 1: (INSP052 Nucleotide sequence exon1)

5       1 ATGAAGAGAG AAAGGGGAGC CCTGTCCAGA GCCTCCAGGG CCCTGCGCCT TGCTCCTTT  
61      61 GTCTACCTTC TTCTGATCCA GACAG

SEQ ID NO 2: (INSP052 polypeptide sequence of Exon 1)

10      1 MKRERGALSR ASRALRLAPF VYLLIQTD  
10

SEQ ID NO 3: (INSP052 Nucleotide sequence exon2)

1       1 ACCCCCTGGA GGGGGTGAAC ATCACCAAGCC CCGTGCAGCCT GATCCATGGC ACCGTGGGGA  
61      61 AGTCGGCTCT GCTTTCTGTG CAGTACAGCA GTACCAGCAG CGACAGGCCT GTAGTGAAGT  
121     121 GGCAGCTGAA GCGGGACAAG CCAGTGACCG TGTTGCAGTC CATTGGCACA GAGGTCATCG  
15      181 GCACCCCTGCG GCCTGACTAT CGAGACCGTA TCCGACTCTT TGAAAATGGC TCCCTGCTTC  
241     241 TCAGCGACCT GCAGCTGGCC GATGAGGGCA CCTATGAGGT CGAGATCTCC ATCACCGACG  
301     301 ACACCTTCAC TGGGGAGAAG ACCATCAACC TTACTGTAGA TG

SEQ ID NO 4: (INSP052 Protein Sequence of Exon 2)

20      1 PLEGVNITSP VRLIHGTVGK SALLSVQYSS TSSDRPVVKW QLKRDKPVTW VQSIGTEVIG  
61      61 TLRPDYRDRI RLFENGSLLL SDLQLADEGT YEVEISITDD TFTGEKTINL TVDV

SEQ ID NO 5: (INSP052 Nucleotide sequence Exon3)

25      1 TGCCCATTTC GAGGCCACAG GTGTTGGTGG CTTCAACCAC TGTGCTGGAG CTCAGCGAGG  
61      61 CCTTCACCTT GAACTGCTCA CATGAGAATG GCACCAAGCC CAGCTACACC TGGCTGAAGG  
121     121 ATGGCAAGCC CCTCCTCAAT GACTCGAGAA TGCTCCTGTC CCCCCACCAA AAGGTGCTCA  
181     181 CCATCACCCG CGTGCTCATG GAGGATGACG ACCTGTACAG CTGCATGGTG GAGAACCCCA  
241     241 TCAGCCAGGG CCGCAGCCTG CCTGTCAAGA TCACCGTATA CA

30    SEQ ID NO 7: (INSP052 Polypeptide sequence of Exon 3)

1    1 PISRPQVLVA STTVLELSEA FTLNCSHENG TKPSYTWLKD GKPLLNDSRM LLSPDQKVLT  
61   61 ITRVLMEDDD LYSCMVENPI SQGRSLPVKI TVYR

SEQ ID NO 7: (INSP052 Nucleotide Sequence Exon 4)

35    1 GAAGAAAGCTC CCTTTACATC ATCTTGCTA CAGGAGGCAT CTTCCCTCCTT GTGACCTTGG

61 TGACAGTCTG TGCCTGCTGG AAACCCTCCA AAAG

SEQ ID NO 8: (INSP052 Polypeptide sequence of Exon 4)

1 RSSLYIILST GGIIFLLVTLV TVCACWKPSK R

5

SEQ ID NO 9: (INSP052 Nucleotide Sequence Exon 5)

1 GAAACAGAAG AAGCTAGAAA AGCAAAACTC CCTGGAATAC ATGGATCAGA ATGATGACCG

61 CCTGAAACCA GAAG

10 SEQ ID NO 10: (INSP052 Polypeptide Sequence Exon 5)

1 KQKKLEKQNS LEYMDQNDDR LKPEA

SEQ ID NO 11: (INSP052 Nucleotide Sequence Exon 6)

1 CAGACACCCCT CCCTCGAAGT GGTGAGCAGG AACGGAAGAA CCCCATGGCA CTCTATATCC

15 61 TGAAGGACAA G

SEQ ID NO 12: (INSP052 Polypeptide Sequence Exon 6)

1 DTLPRSGEQE RKNPMALYIL KDK

20 SEQ ID NO 13: (INSP052 Nucleotide Sequence Exon 7)

1 GACTCCCCGG AGACCGAGGA GAACCCGGCC CCGGAGCCTC GAAGCGCGAC GGAGCCCGGC

61 CCGCCCGGCT ACTCCGTGTC TCCCAGCGTG CCCGGCCGCT CGCCGGGGCT GCCCATCCGC

121 TCTGCCCGCC GCTACCCGCG CTCCCCAGCG CGCTCCCCAG CCACCGGCCG GACACACTCG

181 TCGCCGCCA GGGCCCCGAG CTCGCCCCGGC CGCTCGCGCA GCGCCTCGCG CACACTGCGG

25 241 ACTGCGGGCG TGACACATAAT CCGCGAGCAA GACGAGGCCG GCCCGGTGGA GATCAGCGCC

301 TGA

SEQ ID NO 14: (INSP052 Polypeptide sequence for exon 7)

1 DSPETEENPA PEPRSATEPG PPGYSVSPA V PGRSPGLPIR SARRYPRSPA RSPATGRTHS

30 61 SPPRAPSSPG RSRSASRTL R TAGVHIIREQ DEAGPVEISA

SEQ ID NO :15 (INSP052 Combined Nucleotide sequence exons 1,2,3,4,5,6 and 7)

1 ATGAAGAGAG AAAGGGGAGC CCTGTCCAGA GCCTCCAGGG CCCTGCGCCT TGCTCCTTT

61 GTCTACCTTC TTCTGATCCA GACAGACCCC CTGGAGGGGG TGAACATCAC CAGCCCCGTG

35 121 CGCCTGATCC ATGGCACCGT GGGGAAGTCG GCTCTGCTTT CTGTGCAGTA CAGCAGTACC

181 AGCAGCGACA GGCCTGTAGT GAAGTGGCAG CTGAAGCGGG ACAAGCCAGT GACCGTGGTG

241 CAGTCCATTG GCACAGAGGT CATCGGCACC CTGCGGCCTG ACTATCGAGA CCGTATCCGA  
 301 CTCTTGAAA ATGGCTCCCT GCTTCTCAGC GACCTGCAGC TGGCCGATGA GGGCACCTAT  
 361 GAGGTGAGA TCTCCATCAC CGACGACACC TTCACTGGGG AGAAGACCAT CAACCTTACT  
 421 GTAGATGTGC CCATTCGAG GCCACAGGTG TTGGTGGCTT CAACCACTGT GCTGGAGCTC  
 5 481 AGCGAGGCCT TCACCTTGAA CTGCTCACAT GAGAATGGCA CCAAGCCCAG CTACACCTGG  
 541 CTGAAGGATG GCAAGCCCT CCTCAATGAC TCGAGAATGC TCCTGTCCCC CGACCAAAAG  
 601 GTGCTCACCA TCACCCGCGT GCTCATGGAG GATGACGACC TGTACAGCTG CATGGTGGAG  
 661 AACCCATCA GCCAGGGCCG CAGCCTGCCT GTCAAGATCA CCGTATACAG AAGAAGCTCC  
 721 CTTTACATCA TCTTGTCTAC AGGAGGCATC TTCCCTCCTTG TGACCTTGGT GACAGTCTGT  
 10 781 GCCTGCTGGA AACCCCTCAA AAGGAAACAG AAGAAGCTAG AAAAGCAAA CTCCCTGGAA  
 841 TACATGGATC AGAATGATGA CCGCCTGAAA CCAGAAGCAG ACACCCCTCCC TCGAAGTGGT  
 901 GAGCAGGAAC GGAAGAACCC CATGGCACTC TATATCCTGA AGGACAAGGA CTCCCCGGAG  
 961 ACCGAGGAGA ACCCGGCCCG GGAGCCTCGA AGCGCGACGG AGCCCGGCCG GCCCGGCTAC  
 1021 TCCGTGTCTC CCGCCGTGCC CGGCCGCTCG CCGGGGCTGC CCATCCGCTC TGCCCGCCGC  
 15 1081 TACCCGCGCT CCCCAGCGCG CTCCCCAGCC ACCGGCCGGA CACACTCGTC GCCGCCAGG  
 1141 GCCCCGAGCT CGCCCGGCCG CTCGCGCAGC GCCTCGCGCA CACTGCGGAC TGCGGGCGTG  
 1201 CACATAATCC GCGAGCAAGA CGAGGCCGGC CCGGTGGAGA TCAGCGCCTG A

SEQ ID NO:16 (INSP052 Combined polypeptide sequence for exons 1,2,3,4,5,6 and 7.)

20 1 MKRERGALSR ASRALRLAPF VYLLIQTDP LEGVNITSPV RLIHGTVGKS ALLSVQYSST  
 61 SSDRPVVWKQ LKRDKPVTVV QSIGTEVIGT LRPDYRDRIR LFENGSLLLS DLQLADEGY  
 121 EVEISITDDT FTGEKTINLT DVPISRPQV LVASTTVLEL SEAFTLNC SH ENGTKPSYTW  
 181 LKDGPPLLND SRMLLSPDQK VLTITRVLME DDDLYSCMVE NPISQGRSLP VKITVYRRSS  
 241 LYIILSTGGI FLLVTLVTVC ACWKPSKRQ KKLEKQNSLE YMDQNDDRLK PEAIDLPRSG  
 25 301 EQERKNPMAL YILDKDSDPE TEENPAPEPR SATEPGPPGY SVSPAVPGRS PGLPIRSARR  
 361 YPRSPARSPA TGRTHSSPPR APSSPGRSRS ASRTLRTAGV HIREQDEAG PVEISA

SEQ ID NO:17 (INSP055 Mouse virtual cDNA)

1 ATGAAGAGAG AAAGGGGAGC CCTGTCAAGA GCCTCCAGGG CTCTGCGCCT CTCTCCTTT  
 30 61 GTCTACCTGC TTCTCATCCA GCCAGTCCCC CTGGAGGGGG TGAACATCAC CAGCCAGTA  
 121 CGTCTGATCC ACGGCACAGT GGGGAAGTCG GCCCTGCTT CCGTGCAGTA CAGTAGCACC  
 181 AGCAGCGACA AGCCCGTGGT GAAGTGGCAG CTGAAGCGTG ACAAGCCAGT GACCGTGGTG  
 241 CAGTCTATAG GCACAGAGGT CATTGGCACT CTGCGGCCTG ACTATCGAGA CCGTATCCGG  
 301 CTCTTGAAA ATGGCTCCTT GCTTCTCAGC GACCTGCAGC TGGCGGATGA GGGAACCTAT  
 35 361 GAAGTGGAGA TTTCCATCAC TGACGACACC TTCACCGGGG AGAAGACCAT CAACCTCACC  
 421 GTGGATGTGC CCATTTCAAG GCCGCAGGTA TTAGTGGCTT CAACCACTGT GCTGGAGCTC

481 AGTGAGGCCT TCACCCCTCAA CTGCTCCCAT GAGAATGGCA CCAAGCCTAG CTACACGTGG  
 541 CTGAAGGATG GCAAACCCCT CCTCAATGAC TCCCAGATGC TCCTGTCCCC TGACCAAAAG  
 601 GTGCTCACCA TCACCCGAGT ACTCATGGAA GATGACGACC TGTACAGCTG TGTGGTGGAG  
 661 AACCCCATCA GCCAGGTCCG CAGCCTGCCT GTCAAGATCA CTGTGTATAG AAGAAGCTCC  
 5 721 CTCTATATCA TCTTGTCTAC AGGAGGCATC TTCCCTCCTTG TGACCCCTGGT GACAGTTGT  
 781 GCCTGCTGGA AACCCCTCAAA AAAGTCTAGG AAGAAGAGGA AGTTGGAGAA GCAAAACTCC  
 841 TTGGAATACA TGGATCAGAA TGATGACCGC CTAAAATCAG AAGCAGATAC CCTACCCCGA  
 901 AGTGGAGAAC AGGAGCGGAA GAACCCAATG GCACTCTATA TCCTGAAGGA TAAGGATTCC  
 961 TCAGAGCCAG ATGAAAACCC TGCTACAGAG CCACGGAGCA CCACAGAACCC CGGTCCCCCT  
 10 1021 GGCTACTCCG TGTCGCCGCC CGTGCCTGGC CGCTCTCCGG GGCTTCCCAT CCGCTCAGCC  
 1081 CGCCGCTACC CGCGCTCCCC AGCACGTTCC CCTGCCACTG GCCGGACGCA CACGTCGCCA  
 1141 CCGCGGGCCC CGAGCTCGCC AGGGCGCTCG CGCAGCTCTT CGCGCTCACT CGGGACTGCA  
 1201 GGCGTGCAGA GAATCCGGGA GCAGGGACGAG TCAGGGCAGG TGGAGATCAG TGCCTGA

15 SEQ ID NO:18 (INSP055 Mouse Predicted Protein)

1 MKRERGALSR ASRALRLSPF VYLLIQPVP LEGVNITSPV RLIHGTVGKS ALLSVQYSST  
 61 SSDKPVVKWQ LKRDKPVTVV QSIGTEVIGT LRPDYRDRIR LFENGSLLLS DLQLADEGY  
 121 EVEISITDDT FTGEKTINLT VDVPISRPQV LVASTTVLEL SEAFTLNC SH ENGTKPSYTW  
 181 LKDGPPLLND SRMLLSPDQK VLTITRVLME DDDLYSCVVE NPISQVRSLP VKITVYRRSS  
 20 241 LYIILSTGGI FLLVTLVTVC ACWKPSKKSR KKRKLEKQNS LEYMDQNDDR LKSEADTLPR  
 301 SGEQERKNPM ALYILKDKDS SEPDENPATE PRSTTEPGPP GYSVSPVPG RSPGLPIRSA  
 361 RRYPRSPARS PATGRTHTSP PRAFPSSPGRS RSSSRSLRTA GVQIREQDE SGQVEISA

**CLAIMS**

1. A polypeptide, which polypeptide:

- (i) comprises or consists of the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, or SEQ ID NO:16;
- 5 (ii) is a fragment thereof having the activity of a polypeptide according to (i), or having an antigenic determinant in common with a polypeptide according to (i); or
- (iii) is a functional equivalent of (i) or (ii).

10 2. A polypeptide according to claim 1 which comprises or consists of the amino acid sequence as recited in SEQ ID NO:16.

3. A polypeptide according to claim 2 which consists of the amino acid sequence as recited in SEQ ID NO:16.

4. A polypeptide, which polypeptide:

- 15 (i) comprises or consists of the amino acid sequence as recited in SEQ ID NO:18,
- (ii) is a fragment thereof having the activity of a polypeptide of (i), or having an antigenic determinant in common with a polypeptide of (i); or
- (iii) is a functional equivalent of (i) or (ii).

5. A polypeptide according to claim 4 which consists of the amino acid sequence as recited  
20 in SEQ ID NO:18.

6. A polypeptide which is a functional equivalent according to claim 1 (iii) or claim 4 (iii), characterised in that it is homologous to the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16 or SEQ ID NO:18 and has immunoglobulin domain-  
25 containing cell surface recognition molecule activity.

7. A polypeptide which is a fragment or a functional equivalent according to any one of the preceding claims, which has greater than 84% sequence identity with the amino acid sequence recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18, or

with an active fragment thereof, preferably greater than 85%, 90%, 95%, 98% or 99% sequence identity.

8. A polypeptide which is a functional equivalent as recited in any one of the preceding claims, which exhibits significant structural homology with a polypeptide having the 5 amino acid sequence recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.
9. A polypeptide which is a fragment as recited in any one of the preceding claims having an antigenic determinant in common with the polypeptide of claim 1 (i) or claim 4 (i) 10 which consists of 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more) amino acid residues from the amino acid sequence recited in SEQ ID NO:16 or SEQ ID NO:18.
10. A purified nucleic acid molecule which encodes a polypeptide according to any one of the preceding claims.
11. A purified nucleic acid molecule according to claim 10, which comprises the nucleic 15 acid sequence as recited in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:17 or is a redundant equivalent or fragment thereof.
12. A purified nucleic acid molecule according to claim 11 which consists of the nucleic acid sequence as recited in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID 20 NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:17, or is a redundant equivalent or fragment thereof.
13. A purified nucleic acid molecule which hybridizes under high stringency conditions with a nucleic acid molecule according to any one of claims 10 to 12.
14. A vector comprising a nucleic acid molecule as recited in any one of claims 10 to 13.
- 25 15. A host cell transformed with a vector according to claim 14.
16. A ligand which binds specifically to, and which preferably inhibits the activity of a polypeptide according to any one of claims 1 to 9.
17. A ligand according to claim 16, which is an antibody.
18. A compound that either increases or decreases the level of expression or activity of a 30 polypeptide according to any one of claims 1 to 9.

19. A compound according to claim 18 that binds to a polypeptide according to any one of claims 1 to 9 without inducing any of the biological effects of the polypeptide.
20. A compound according to claim 19, which is a natural or modified substrate, ligand, enzyme, receptor or structural or functional mimetic.
- 5 21. A polypeptide according to any one of claims 1 to 9, a nucleic acid molecule according to any one of claims 10 to 13, a vector according to claim 14, a ligand according to claim 16 or claim 17, or a compound according to any one of claims 18 to 20, for use in therapy or diagnosis of disease.
- 10 22. A method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to any one of claims 1 to 9, or assessing the activity of a polypeptide according to any one of claims 1 to 9, in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease.
23. A method according to claim 22 that is carried out *in vitro*.
- 15 24. A method according to claim 22 or claim 23, which comprises the steps of: (a) contacting a ligand according to claim 16 or claim 17 with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.
25. A method according to claim 22 or claim 23, comprising the steps of:
  - 20 a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule according to any one of claims 10 to 13 and the probe;
  - b) contacting a control sample with said probe under the same conditions used in step a); and
  - 25 c) detecting the presence of hybrid complexes in said samples; wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.
26. A method according to claim 22 or claim 23, comprising:
  - 30 a) contacting a sample of nucleic acid from tissue of the patient with a nucleic acid primer under stringent conditions that allow the formation of a hybrid complex

between a nucleic acid molecule according to any one of claims 10 to 13 and the primer;

b) contacting a control sample with said primer under the same conditions used in step a); and

5 c) amplifying the sampled nucleic acid; and

d) detecting the level of amplified nucleic acid from both patient and control samples; wherein detection of levels of the amplified nucleic acid in the patient sample that differ significantly from levels of the amplified nucleic acid in the control sample is indicative of disease.

10 27. A method according to claim 22 or claim 23 comprising:

a) obtaining a tissue sample from a patient being tested for disease;

b) isolating a nucleic acid molecule according to any one of claims 10 to 13 from said tissue sample; and

c) diagnosing the patient for disease by detecting the presence of a mutation which  
15 is associated with disease in the nucleic acid molecule as an indication of the disease.

28. The method of claim 27, further comprising amplifying the nucleic acid molecule to form an amplified product and detecting the presence or absence of a mutation in the amplified product.

20 29. The method of claim 27 or claim 28, wherein the presence or absence of the mutation in the patient is detected by contacting said nucleic acid molecule with a nucleic acid probe that hybridises to said nucleic acid molecule under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a  
25 mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation.

30. A method according to any one of claims 22 to 29, wherein said disease is a cell proliferative disorder, an autoimmune/inflammatory disorder, a cardiovascular disorder, a neurological disorder, a psychiatric disorder, a developmental disorder, a

genetic disorder, a metabolic disorder, an infection or other pathological condition.

31. Use of a polypeptide according to any one of claims 1 to 9 as an immunoglobulin domain-containing cell surface recognition molecule.
32. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 9, a nucleic acid molecule according to any one of claims 10 to 13, a vector according to claim 14, a ligand according to claim 16 or claim 17, or a compound according to any one of claims 18 to 20.  
5
33. A vaccine composition comprising a polypeptide according to any one of claims 1 to 9 or a nucleic acid molecule according to any one of claims 10 to 13.
- 10 34. A polypeptide according to any one of claims 1 to 9, a nucleic acid molecule according to any one of claims 10 to 13, a vector according to claim 15, a ligand according to claim 16 or claim 17, or a compound according to any one of claims 18 to 20 or a pharmaceutical composition of claim 32, for use in the manufacture of a medicament for the treatment of a disease selected from the group consisting of: cell proliferative disorders, autoimmune/inflammatory disorders, cardiovascular disorders, neurological and psychiatric disorders, developmental disorders, genetic disorders, metabolic disorders, infections and other pathological conditions.  
15
35. A method of treating a disease in a patient, comprising administering to the patient a polypeptide according to any one of claims 1 to 9, a nucleic acid molecule according to any one of claims 10 to 13, a vector according to claim 14, a ligand according to claim 16 or claim 17, or a compound according to any one of claims 18 to 20 or a pharmaceutical composition of claim 32.  
20
36. A method according to claim 35, wherein, for diseases in which the expression of the natural gene or the activity of the polypeptide is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, ligand, compound or composition administered to the patient is an agonist.  
25
37. A method according to claim 35, wherein, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, ligand, compound or composition administered to the  
30

patient is an antagonist.

38. A method of monitoring the therapeutic treatment of disease in a patient, comprising monitoring over a period of time the level of expression or activity of a polypeptide according to any one of claims 1 to 9, or the level of expression of a nucleic acid molecule according to any one of claims 10 to 13 in tissue from said patient, wherein altering said level of expression or activity over the period of time towards a control level is indicative of regression of said disease.  
5
39. A method for the identification of a compound that is effective in the treatment and/or diagnosis of disease, comprising contacting a polypeptide according to any one of claims 1 to 9, or a nucleic acid molecule according to any one of claims 10 to 13 with one or more compounds suspected of possessing binding affinity for said polypeptide or nucleic acid molecule, and selecting a compound that binds specifically to said nucleic acid molecule or polypeptide.  
10
40. A kit useful for diagnosing disease comprising a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to any one of claims 10 to 13; a second container containing primers useful for amplifying said nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease.  
15
41. The kit of claim 40, further comprising a third container holding an agent for digesting unhybridised RNA.  
20
42. A kit comprising an array of nucleic acid molecules, at least one of which is a nucleic acid molecule according to any one of claims 10 to 13.
43. A kit comprising one or more antibodies that bind to a polypeptide as recited in any one of claims 1 to 9, and a reagent useful for the detection of a binding reaction between said antibody and said polypeptide.  
25
44. A transgenic or knockout non-human animal that has been transformed to express higher, lower or absent levels of a polypeptide according to any one of claims 1 to 9.
45. A method for screening for a compound effective to treat disease, by contacting a non-human transgenic animal according to claim 44 with a candidate compound and determining the effect of the compound on the disease of the animal.  
30

**Figure 1: NCBI non-redundant database BLAST hits against INSP052**

BLASTP 2.2.1 [Jul-12-2001]

5

Reference: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

10

Query= genscan2

(416 letters)

Database: ncbi-nr

15

897,014 sequences; 280,886,335 total letters

Searching.....done

		Score	E
	Sequences producing significant alignments:	(bits)	Value
20	pir  JC1512 biliary glycoprotein H - mouse	81	2e-14
	pir  JC1506 biliary glycoprotein B - mouse	81	2e-14
	pir  A39037 carcinoembryonic antigen mmCGM2 precursor - mouse >g...	79	9e-14
25	ref NP_036056.1  (NM_011926) CEA-related cell adhesion molecule ...	79	9e-14
	pir  JC1509 biliary glycoprotein E - mouse	73	5e-12
	ref NP_001758.1  (NM_001767) CD2 antigen (p50), sheep red blood ...	73	6e-12
	ref NP_113943.1  (NM_031755) carcinoembryonic antigen-related ce...	72	8e-12
	pir  RWHUC2 T-cell surface glycoprotein CD2 precursor - human >g...	72	8e-12
30	gb AAA51946.1  (M16336) CD2 surface antigen [Homo sapiens]	72	8e-12
	ref NP_291021.1  (NM_033543) hypothetical protein R29124_1 [Homo...	72	1e-11
	pir  JC1507 biliary glycoprotein C - mouse	71	2e-11
	emb CAA47697.1  (X67280) biliary glycoprotein [Mus musculus]	71	2e-11
	pir  S34338 biliary glycoprotein F - mouse >gi 312586 emb CAA476...	71	2e-11
35	pir  JC1511 biliary glycoprotein G - mouse	71	2e-11

**Figure 2: Alignment between INSP052 with its top hit, biliary glycoprotein H (mouse)**

>pir||JC1512 biliary glycoprotein H - mouse

Length = 341

5

Score = 80.9 bits (198), Expect = 2e-14

Identities = 54/168 (32%), Positives = 86/168 (51%), Gaps = 9/168 (5%)

Query: 73 RDKPVTVVQSIGTEVIGTLR----PDYRDRIRLFENGSLLLSDLQLADEGTYEVEISITD 128

10

+ PV+ I +V GT + P + R ++ NGSL+ + + D G Y +E+ TD

Sbjct: 69 KGNPVSTNAEIVHQVTGTNKTGPAHSGRET VYSNGSLLIQRVTVKDTGVYTIEM--TD 126

Query: 129 DTFTG-EKTINLTVDPVISRPQVLVASTTVLELSEAFTLNC SHENGTKPSYT WLKDGPL 187

15

+ F E T+ V P+++P + V +TTV EL ++ TL C N + WL + + L

Sbjct: 127 ENFRRTEATVQFHVHQPVTLQPSLQVTNTVKEL-DSVLTCL-SNDIGANIQWLFNSQSL 184

Query: 188 LNDSRMLLSPDQKVLTITRVL MEDDDLYSCMVENPISQGRSLPVKITV 235

RM LS + +L I + ED Y C + NP+S RS +K+ +

Sbjct: 185 QLTERMTLSQNNISILRIDPIKREDAGEYQCEISNPVSVKRSNSIKLDI 232

20

**Figure 3: Top ten NCBI non-redundant database BLAST hits against INSP055**

			Score	E
		Sequences producing significant alignments:	(bits)	Value
5				
	gi 483306 pir  JC1506	biliary glycoprotein B - mouse	79	1e-13
	gi 111207 pir  A39037	carcinoembryonic antigen mmCGM2 precu...	77	3e-13
	gi 483312 pir  JC1512	biliary glycoprotein H - mouse	77	4e-13
	gi 13937381 ref NP_036056.1	(NM_011926) CEA-rélated cell a...	75	1e-12
10	gi 228710 prf  1809184A	pregnancy-specific glycoprotein [Ra...	70	5e-11
	gi 483307 pir  JC1507	biliary glycoprotein C - mouse	70	6e-11
	gi 16117775 ref NP_291021.1	(NM_033543) hypothetical prote...	69	8e-11
	gi 483309 pir  JC1509	biliary glycoprotein E - mouse	69	9e-11
	gi 312582 emb CAA47695.1	(X67278) biliary glycoprotein [Mu...	69	1e-10
15	gi 483311 pir  JC1511	biliary glycoprotein G - mouse	68	2e-10

**Figure 4: Alignment between INSP055 with its top hit, biliary glycoprotein B - mouse**

```

>gi|483306|pir||JC1506 biliary glycoprotein B - mouse
Length = 278
5
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10      + PV+     I +V GT +     P +   R ++ NGSLL+ + + D G Y +E +TD
Sbjct: 69 KGNPVSTNAEIVHQVTGPNKTTGPAHSGRET VYNSNGSLLIQRVTVKDTGVYTIE--MTD 126

Query: 129 DTF-TGEKTINLTVDVPISRQQLVASTTVLELSEAFTLNC SHENGTKPSYTWLKDGPPL 187
      + F     E T+     V P+++P + V +TTV EL ++ TL C   N      + WL + + L
15 Sbjct: 127 ENFRRTEATVQFHVHQPVTLQVNTTVKEL-DSVLTCL-SNDIGANIQWLFNSQSL 184

Query: 188 LNDSRMLLSPDQKVLTITRVL MEDDDLYSCVVENPISQVRSLPVKITV 235
      RM LS + +L I + ED Y C + NP+S RS +K+ +
Sbjct: 185 QLTERMTLSQNN SILRIDPIKREDAGEYQCEISNPVSVKRSNSIKLDI 232
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BLASTP 2.2.1 [Jul-12-2001]

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30	gb AAA51946.1  (M16336) CD2 surface antigen [Homo sapiens]	72	8e-12
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**Figure 2: Alignment between INSP052 with its top hit, biliary glycoprotein H (mouse)**

>pir|JC1512 biliary glycoprotein H - mouse

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+ PV+ I +V GT + P + R ++ NGSLL+ + + D G Y +E+ TD

Sbjct: 69 KGNPVSTNAEIVHQVTGTNKTGPAHSGRETVYSNGSLLIQRVTVKDTGVYTIEM--TD 126

Query: 129 DTFTG-EKTINLTVDVPISRPQVLVASTTVLELSEAFTLNCSHENGTKPSYTWLKDGPL 187

+ F E T+ V P+++P + V +TTV EL ++ TL C N + WL + + L

15

Sbjct: 127 ENFRRTEATVQFHVHQPVTLQPSLQVTNTTVKEL-DSVTLTCL-SNDIGANIQWLFNSQL 184

Query: 188 LNDSRMLLSPDQKVLTITRVLMEDDDLYSCMVENPISQGRSLPVKITV 235

RM LS + +L I + ED Y C + NP+S RS +K+ +

Sbjct: 185 QLTERMTLSQNNISILRIDPIKREDAGEYQCEISNPVSVKRSNSIKLDI 232

20

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		Score	E
		(bits)	Value
Sequences producing significant alignments:			
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	gi 111207 pir  A39037	carcinoembryonic antigen mmCGM2 precu...	77 3e-13
	gi 483312 pir  JC1512	biliary glycoprotein H - mouse	77 4e-13
	gi 13937381 ref NP_036056.1	(NM_011926) CEA-related cell a...	75 1e-12
10	gi 228710 prf  1809184A	pregnancy-specific glycoprotein [Ra...	70 5e-11
	gi 483307 pir  JC1507	biliary glycoprotein C - mouse	70 6e-11
	gi 16117775 ref NP_291021.1	(NM_033543) hypothetical prote...	69 8e-11
	gi 483309 pir  JC1509	biliary glycoprotein E - mouse	69 9e-11
	gi 312582 emb CAA47695.1	(X67278) biliary glycoprotein [Mu...	69 1e-10
15	gi 483311 pir  JC1511	biliary glycoprotein G - mouse	68 2e-10

**Figure 4: Alignment between INSP055 with its top hit, biliary glycoprotein B - mouse**

>gi|483306|pir||JC1506 biliary glycoprotein B - mouse

Length = 278

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Score = 78.6 bits (192), Expect = 1e-13

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10

+ PV+ I +V GT + P + R ++ NGSLL+ + + D G Y +E +TD

Sbjct: 69 KGNPVSTNAEIVHQVTGTNKTTPAHSGRETVYSNGSLLIQRVTVKDTGVYTIE--MTD 126

Query: 129 DTF-TGEKTINLTVDVPISRPQVLVASTTVLELSEAFTLNCSHENGTKPSYTWLKDGPL 187

+ F E T+ V P+++P + V +TTV EL ++ TL C N + WL + + L

15

Sbjct: 127 ENFRRTEATVQFHVHQPVTLQVTNTTVKEL-DSVTLTCL-SNDIGANIQWLFNSQSL 184

Query: 188 LNDSRMLLSPDQKVLTITRVLMEDDDLYSCVVENPISQVRSLPVKITV 235

RM LS + +L I + ED Y C + NP+S RS +K+ +

Sbjct: 185 QLTERMTLSQNNISILRIDPIKREDAGEYQCEISNPVSVKRSNSIKLDI 232

20